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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

PHYSIOLOGY

REPRESENTATION OF INTERNAL ORGANS IN THE CEREBRAL CORTEX AND CEREBELLUM OF CATS AND DOGS

COMMUNICATION 2: PRIMARY BIOELECTRIC REACTIONS OF THE CEREBRAL CORTEX TO STIMULATION OF INTERNAL ORGANS

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It was shown in previous communications [1, 2] that the pelvic nerve in cats and dogs was represented in the cerebral cortex with definite localization. In cats one zone is situated on g. cruciatus, another on g. ectosylvius ant., both zones being bilateral (Fig. 2, scheme 1). Primary responses appear in these zones on both hemispheres on stimulation of the pelvic nerve with single shocks.

Similar reactions in corresponding areas of the cortex may be detected on electric stimulation of other afferent nerves from internal organs, such as the vagus and splanchnic (Amassian, Dell et al).

There is now no doubt concerning the possibility of primary bioelectric reaction occurring in the cerebral cortex in response to stimulation of any afferent nerve. At the same time, the question of primary responses to adequate stimulation of the peripheral terminations of analysors has not been sufficiently investigated. So far it has proved possible to obtain cortical primary responses to adequate stimulation of the retina, the organ of Corti and cutaneous receptors [4].

Almost nothing has so far been discovered about the possibility of obtaining primary responses to visceral stimulation. Thus the present author found only one reference in the literature, in the work of V. Amassian, to a bioelectric reaction, which he considered as a primary response, recorded from the contralateral zone of cortical representation of the splanchnic nerve on stimulation of intestinal mesentery in the cat with a brief single mechanical stimulus [5].

No data with reference to other viscera were available.

In the course of mapping the zones of cortical representation of various internal organs we have found that mechanical stimulation of the bladder, rectum and uterus gives rise to primary responses in the cerebral cortex of cats and dogs; these are recorded from zones in which primary responses to stimulation of the pelvic nerve were recorded [1]. In that communication certain characteristics of the responses to stimulation of internal organs were also given.

Subsequently T. E. Orlova [3] reported that when air was insufflated into the bile ducts in dogs it was possible, in part of the experiments, to detect a "local" reaction in the premotor cortical zone which proceeded in accordance with the "primary effect" type of reaction.

The obvious inadequacy of data concerning this question led to this more detailed investigation.

EXPERIMENTAL

Short experiments under nembutal or chloralose anesthesia were performed on 48 cats and 6 dogs; in these experiments the appearance of primary responses in definite cortical zones following stimulation of the bladder, uterus, rectum, stomach and pericardium was studied. Several organs were subjected to stimulation in one and the same experiment. The bladder, rectum and stomach were stimulated by inflation of a fine rubber balloon inserted in their lumen; the uterus and pericardium were stimulated by pulling on silk ligatures attached to them or by touching with a sharp-pointed glass rod.

On stimulation of the bladder the maximal pressure in the balloon, recorded by a kymograph, was equal to 50 mm of mercury. In order to avoid artefacts which arise whenever the distended organ comes into contact with the abdominal wall or the surface of the skin, the bladder was withdrawn from the widely opened abdominal cavity and suspended by ligatures from an insulated stand, while the edges of the incision were widely separated by means of silk ligatures. In addition to the inserted balloon, a cannula was tied into the floor of the bladder which connected its cavity with two recording devices by means of a system filled with warm physiologic solution. The first of the recording devices registered the periodic contractions of the bladder on a kymograph, while the second recorded the same on a cine-film simultaneously with a recording of bioelectric processes (Fig. 1 and 2).

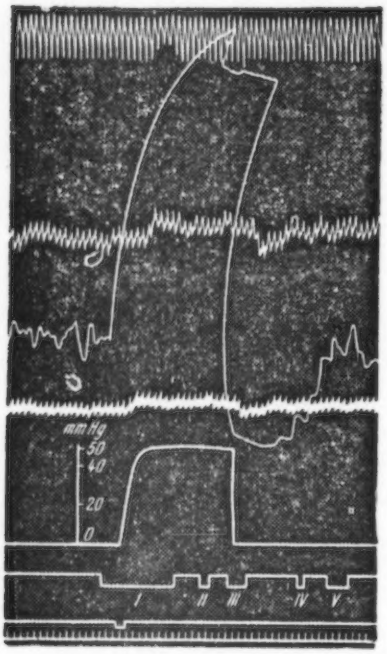

Figure 1. Stimulation of the bladder by inflation of an inserted rubber balloon. Cat. Chloralose anesthesia of medium depth. Records from above down: 1) respiration; 2) arterial pressure shown by mercury manometer; 3) pressure within the bladder shown by tonometer; 4) arterial pressure shown by tonometer; 5) pressure in rubber balloon shown by mercury manometer; 6) moments of recording the oscillograms given in Fig. 2, A, B, C, D, E; 7) beginning of stimulation; 8) time marker (5 seconds).

One of the small branches of the pelvic nerve was dissected and its peripheral end placed on a pair of silver recording electrodes with an interelectrode distance of 5 mm. The electrodes were mounted on a small block of Plexiglas which was attached to the surface of the bladder; this diminished the difficulties usually attendant on displacement of the nerve on the electrodes with changes in the volume of the organ. These electrodes recorded afferent impulses arising in the pelvic nerve on distention of the bladder walls. During the whole experiment measures were taken to protect the surface of the bladder from drying and cooling.

In order to exclude possible reflex contraction of muscles the animals were immobilized in a number of experiments by intravenous administration of "diplacin" (0.5 ml 2% solution per 1 kg body weight). The monopolar method of recording cerebral biopotentials has been described in detail in a previous communication.

The frequency characteristic of the amplifier was linear within the limits from 10 to 500 cps during the recording of afferent impulses and from 5 to 300 cps during the recording of cortical biopotentials.

RESULTS

All the experiments gave, in general, similar results, viz: stimulation of mechanoreceptors of the organs mentioned led to the appearance of primary responses in the cerebral cortex which, in the case of the bladder,

rectum and uterus, were recorded both from the first and second zones of cortical representation of the pelvic nerve. Stimulation of the stomach and pericardium evoked the appearance of primary responses in the first and second regions of general somatic sensation [2] in zones adjacent to the cortical projections of the pelvic and splanchnic nerves. Results of one of these experiments are presented in Figs. 1 and 2.

Comparison of the kymogram with the oscillograms indicates that the moment of distention of the bladder walls is accompanied by the appearance of afferent impulses in the pelvic nerve and of bioelectric reaction in the cerebral cortex (Fig. 1, 1; Fig. 2, A) in the first zone of cortical representation of the pelvic nerve (Fig. 2, scheme 2, C). The question arises as to whether it is possible to regard this bioelectric reaction of the cortex as a primary response to the stimulation of the bladder mechanoreceptors. One of the main signs of a primary response is its latent period. When the pelvic or splanchnic nerve is stimulated by single electric stimulus the length of the latent period usually fluctuates between 6 and 12 milliseconds, whereas in the cortical reaction under consideration the latent period is 30 milliseconds. This difference in the length of the latent periods becomes explicable when it is taken into account that primary responses, obtained on adequate stimulation of receptors, have a longer latent period. For example, the latent period of the primary response to electric stimulation of the optic nerve equals 2.3 milliseconds, while on adequate stimulation of the retina (by illumination of the eye) it fluctuates between 17 and 25 milliseconds [6]. This can be explained by the time required to excite the receptors in the latter case. However, if orientation is based not on the mechanical marker of the beginning of stimulation with its degree of inertia, but on the appearance of afferent impulses as a signal of the beginning of stimulation (such impulses show that some of the receptors have already been excited) even then the value of the latent period exceeds the time necessary for the impulses to reach the cerebral cortex. All this leads to the hypothesis that in order for primary cortical reaction to occur in response to stimulation of internal organ receptors it is necessary that a certain threshold quantity of afferent impulses reach the sensory nuclei of the thalamus. The afferent impulses cause a gradual growth of excitation in the thalamic neurons culminating in a discharge into the cellular layers of the cortex, this latter event being accompanied by the appearance of the primary reaction.

In essence this is similar to electric stimulation of sensory nerves: weak single stimuli produce excitation in the nerve but do not evoke a primary response in the cortex.

These views find a certain analogy in A. I. Roitbak's [4] interpretation of the causes of the increase in amplitude of the positive potential of the primary response on increased peripheral stimulation. Thus, the long latent period of the bioelectric reaction under consideration in the present work cannot be considered as an obstacle to interpreting it as a primary response. Nor is it contradicted by its other characteristics. The somewhat increased duration of its phases can be explained by nonsynchrony of afferent impulses. In those cases where stimulation is associated with a more powerful and synchronous burst of afferent impulses the duration of the phases of the primary response approaches that of responses to nerve stimulation (Fig. 2, F). Primary reactions to stimulation of internal organs, similarly to primary responses to nerve stimulation, are localized. Thus, when the bladder is stimulated they arise in the zones of pelvic nerve representation (Fig. 2, A record at point C, scheme 2) and do not appear in other regions of the cortex. In cortical areas at a distance from the zones of pelvic nerve representation a generalized reaction is observed, expressed by changes in the basic rhythm of bioelectric activity. In the experience of the present author this is reflected in some depression of the basic rhythm. Analogous picture is observed on stimulation of the rectum (Fig. 3). In some cases, however, stimulation of internal organs led to primary responses which had a fairly brief latent period (12-13 milliseconds) following the appearance of afferent impulses (Fig. 2, G). Evidently this occurs in those cases where the excitation of the corresponding thalamic sensory nuclei neurons is such that the brief burst of afferent impulses suffices to produce their discharge into the cellular structures of the cortex.

Such a state of thalamic neurons can be determined by a continuous inflow of afferent impulses from other visceral receptive fields.

If it is supposed that thalamic neurons are capable of summating stimuli ascending from the viscera along afferent pathways and of discharging periodically into the cortex then the bioelectric complexes arising in the cerebral cortex in response to continuing stimulation (Fig. 1, II, Fig. 2, B) may be regarded as individual primary responses following each other in a way in which they would have arisen in the case of rhythmic stimulation of receptors or afferent nerves.

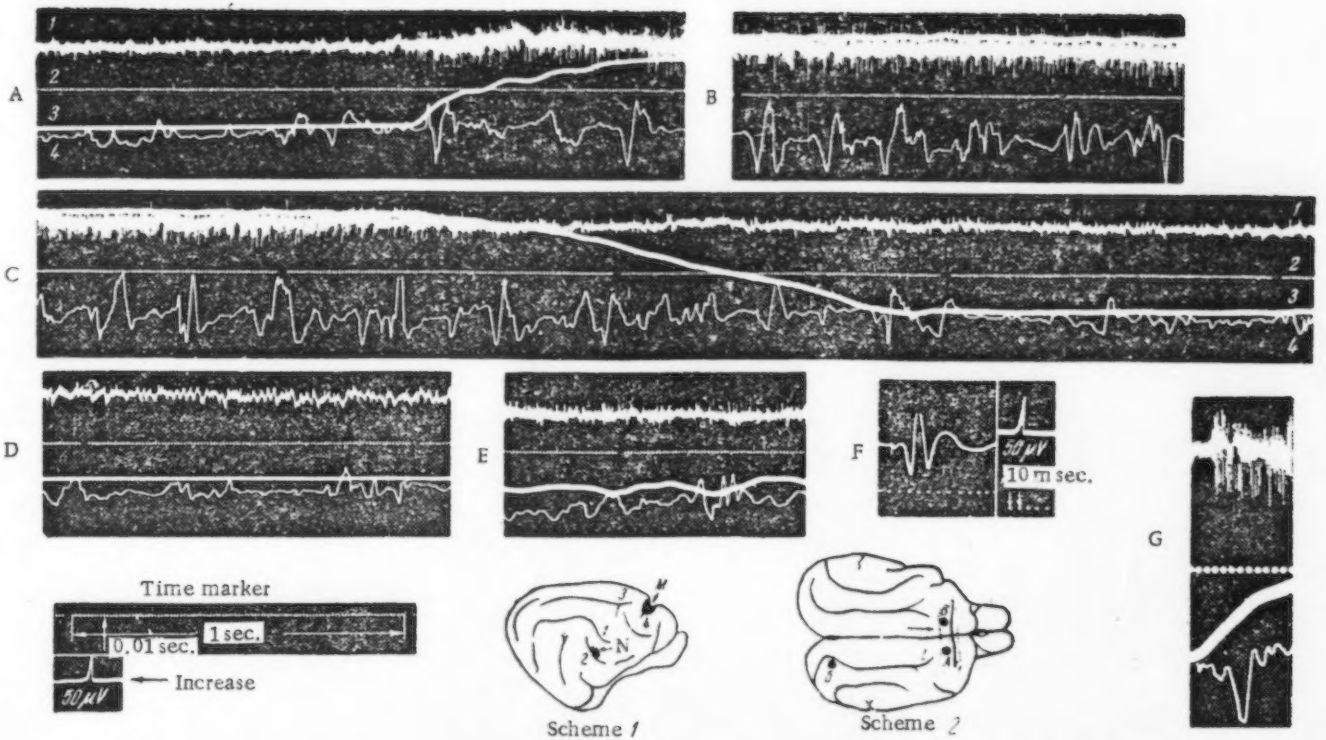

Figure 2. Appearance of primary responses in the cerebral cortex of cat on stimulation of the bladder by inflation of an inserted rubber balloon. Records in all oscillograms from above down: 1) afferent impulses in n. pelvicus; 2) time marker (1 second and 0.01 second); 3) changes in pressure within the bladder; 4) cerebral cortical biopotentials. Scheme 1: M – first and N – second zones of cortical respresentation of n. pelvicus; 1) s. suprasylvius ant.; 2) s. ectosylvius ant.; 3) s. ansatus; 4) s. cruciatus. Scheme 2: 1) s. cruciatus; 2) s. ansatus; F) primary cortical response to stimulation of n. pelvicus by a single shock; G) primary response in the first zone of pelvic nerve representation on stimulation of the bladder possessing a short latent period (12 milliseconds) following the appearance of afferent impulses.

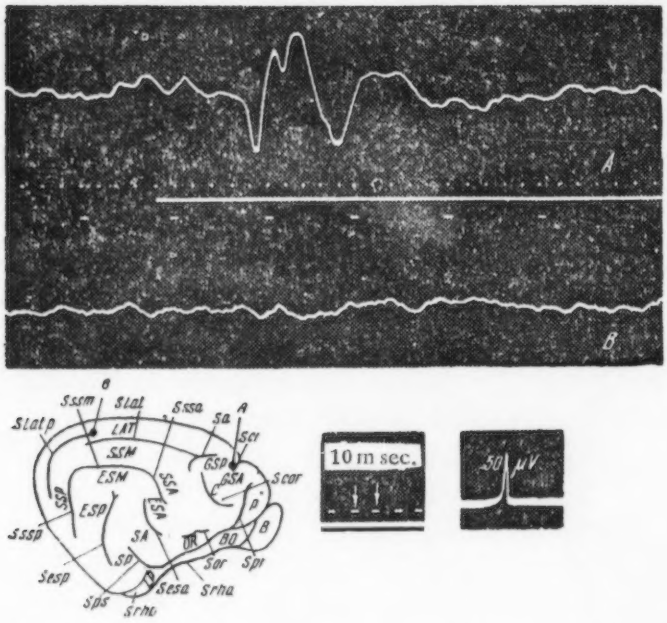

Figure 3. Appearance of primary response in the first zone of cortical representation of the pelvic nerve (A) on stimulation of the rectum and its absence from other cortical areas (B). Cat. Nembutal anesthesia of considerable depth. Animal immobilized by intravenous administration of "diplacin" (artificial respiration). Below at left surface map of the cat's brain with recording electrode positions marked A and B. Continuous line in the center of the oscillogram marks the beginning of inflation of rubber balloon in rectum. Maximal pressure of air in balloon equalled 40 mm of mercury.

The proposed hypothesis is not contradicted by changes in configuration of the primary complexes (Fig. 2, B and C).

The pattern of changes in the configuration of primary responses observed by A. I. Roitbak [4] on rhythmic electric stimulation of cutaneous receptors and rhythmic stimulation of the sciatic nerve appears to resemble the pattern described here. We observed similar results on rhythmic stimulation of the pelvic and splanchnic nerves.

Further analysis of the data obtained draws attention to the fact that the appearance of primary complexes in the cortex continues for some time after cessation of stimulation of the bladder (Fig. 1, III, Fig. 2, C).

Evidently the excitation of thalamic neurons under the influence of afferent impulses persists for some time following their termination and thus determines the periodic, gradually weakening, discharges into the cortex. The possibility is not excluded that this excitation is maintained by afferent stimuli arriving from other organs as well.

Afferent impulses in the pelvic nerve appear anew when the relaxation of the bladder wall (Fig. 1, IV, Fig. 2, D) is replaced by its tension, i.e, at the moment of restoration of its rhythmic contractions. Their appearance is accompanied by primary complexes in the cortex (Fig. 1, V, Fig. 2, E). This may indicate that the normal activity of internal organs (for example contraction of the rectum or stomach, stretching of the bladder wall) is capable of eliciting primary responses in the cerebral cortex.

It is quite probable that the periodic bursts of slow potentials of large amplitude observed in the premotor cortical area and in the second general somatic sensory area, which arise without any preliminary experimental stimulation, are primary complexes determined by the activity of internal organs.

The primary responses which arise in the cortical areas mentioned above on stimulation of the uterus, stomach and pericardium are similar to those which arise in response to stimulation of the bladder or rectum and there is thus no need for a special description of them.

SUMMARY

Experiments on cats and dogs have shown that stimulation of the urinary bladder, colon and uterus elicits a primary reaction in the I and II zones of cortical representation of the pelvic nerve. Stimulation of the stomach and pericardium elicits primary reactions in the I and II areas of general sensitivity, bordering the area of representation of the pelvic and splanchnic nerves. If the afferent impulses of the pelvic nerve during dilatation of the urinary bladder are compared with the cortical potentials one might suppose that summation of excitations takes place in the thalamic neurons, while the bursts of slow potentials and great amplitude, registered during prolonged stimulation of the bladder, might be regarded as a series of primary complexes, originating in the IV cortical layer after thalamic discharges.

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** Original Russian pagination. See C. B. translation.

INVESTIGATION OF THE FUNCTION OF A SINGLE AORTIC BARORECEPTOR

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The afferent activity of the aortic nerve was studied electrophysiologically by Alvarets-Buya [1], P. K. Anokhin and A. I. Shumilina [3]. The oscillogram of the action potentials of the whole aortic nerve presents a picture of the summated activity of many nerve fibers and baroreceptors associated with them. In order to study certain questions concerning the functioning of baroreceptors of the aortic arch it is essential to have a single nerve fiber preparation, associated with a single baroreceptor.

The present author is aware of only one work, by D. W. Bronk [7], devoted to investigation of a single aortic baroreceptor. The usual method of obtaining a single nerve fiber preparation consists of dissecting it from the nerve trunk. This method is rarely successful, especially in the case of warm-blooded animals.

Completely satisfactory preparations of a single baroreceptor with the corresponding single nerve fiber were obtained by the present author on rabbits using the heterogeneous nerve anastomosis method as described by P. K. Anokhin [2].

The central end of the phrenic nerve was sutured to the peripheral end of the sectioned aortic nerve. It is known that the phrenic nerve contains, in addition to the main mass of motor fibers, a small number of sensory fibers which are predominantly proprioceptive [5, 6, 9]. After suturing, the nerve fibers of the phrenic nerve grow into the sheaths of the fibers in the peripheral portion of the aortic nerve. At the periphery fully functional connections with receptors are formed only by sensory fibers. This was shown on other models by V. F. Lashkov [4] and P. Weiss [10].

EXPERIMENTAL METHODS AND RESULTS

Experiments were performed on 6 rabbits. 3-4 weeks after operation the nerves regenerated and preparations of a single nerve fiber or of several nerve fibers were obtained; in the latter case the number of fibers

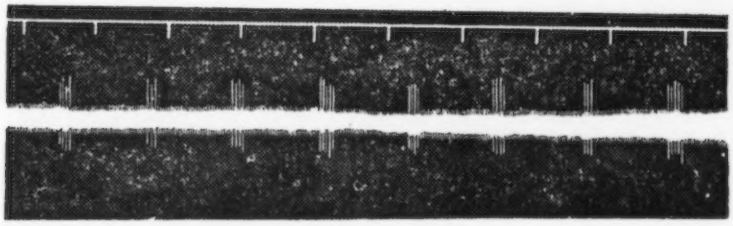
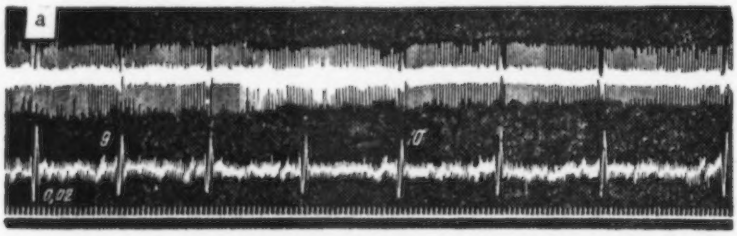


Figure 1. Action potentials of a single fiber of the aortic nerve with normal blood pressure. Time marker (0.2 seconds).

could be readily reduced to one by fractional damage under oscillographic control. When a burst of action potentials appeared below the anastomosis after each systole and the individual oscillations constituting the burst were of equal amplitude the biocurrents were considered to emanate from a single nerve fiber. The action potentials were recorded by means of an A. C. amplifier and oscillograph. Biocurrents characteristic of a single nerve fiber (of equal amplitude) were obtained in all 6 cases of anastomosis.

Figure 1 shows the oscillogram of single nerve fiber action potentials with normal blood pressure. The bursts consist of oscillations equal in amplitude which appear 0.04 seconds after a systole. Each burst contains



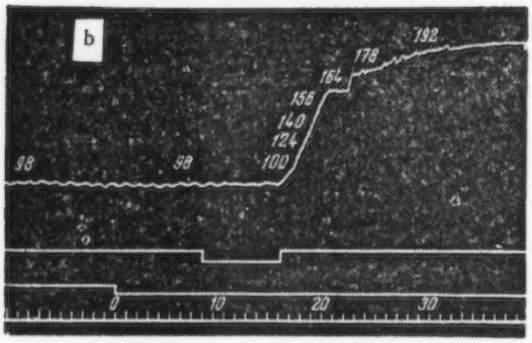


Figure 2. Changes in single nerve fiber action potential discharges in the aortic nerve on raising the blood pressure. Records from above down: a) aortic nerve action potentials; electrocardiogram; time marker (0.02 seconds) b) kymogram of blood pressure; mark denoting injection of adrenaline; mark denoting beginning of action potential recording; time marker (1 second).

1-5 action potentials. The following alternation of the number of discharges in a burst has been noted: 3 – 4 – 4 – 5 – 4 – 3. This evidently reflects respiratory undulations, since the period of the cycle equals the duration of the respiratory undulation on the blood pressure curve, i. e, 1.5 second.

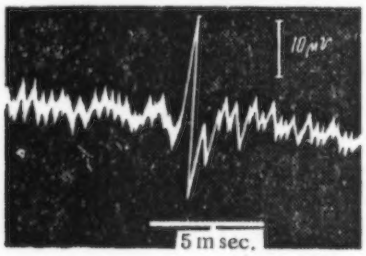


Figure 3. Action potential of a single aortic nerve fiber in the rabbit.

Figure 2 shows the oscillogram of the same nerve fiber 10-12 seconds after intravenous injection of 1.2 mg adrenaline. Below that is given the kymogram showing the level of blood pressure. 4 $\frac{1}{2}$ seconds after injection of adrenaline the minimal number of impulses in a discharge increased from 3 to 5 and oscillations at the end of diastole appeared corresponding to the dicrotic wave.

As the blood pressure rose the burst became

more extended, the frequency of the oscillations constituting the burst became higher, and the burst eventually occupied the whole diastolic interval. The latent period of the burst diminished from 0.04 to 0.02 seconds with the rise of blood pressure. At the beginning of the burst the oscillations had a frequency of 150 cps, reaching a maximum of 250 cps after 60 milliseconds and dropping to 50 cps at the end of the burst.

Characteristically, the amplitude of the action potentials decreased at times by half as compared with the initial amplitude as the rhythm increased (Fig. 3).

The oscillogram of a single action potential of the preparation under investigation as recorded with high speed makes it possible to characterize the single aortic nerve fiber biocurrent in more detail (Fig. 3). The sharpness of the first phase of the wave must be noted, as it is generally characteristic for single nerve fiber action potentials [8]. The duration of the action potential of both phases is 1 millisecond. The amplitude of the action potential is about 30 μv.

The method employed allows more single and adequate conditions for the investigation of the functioning of a single aortic baroreceptor under conditions of natural stimulation.

SUMMARY

An original method of obtaining preparations of isolated aortic nerve fibres by anastomosing the phrenic and aortic nerves is described. Discharge of impulses from the isolated aortic nerve fibre consist of biopotentials of an alternating rhythm. At the beginning of the discharge the frequency of impulsation is high, later it falls down to zero. In case of persistent hypertension impulsations of succeeding bursts blend together forming an unbroken line, with increasing frequency in rhythm of every newly-appearing burst.

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• In Russian

THE ROLE OF INTEROCEPTORS IN THE REGULATION OF OXYGEN SATURATION OF ARTERIAL BLOOD

COMMUNICATION I: THE ROLE OF THE CAROTID SINUS ZONES IN THE REGULATION OF OXYGEN SATURATION OF ARTERIAL BLOOD IN HYPOXIA

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The degree of oxygen saturation of arterial blood is known to be closely related to the partial pressure of oxygen in the surrounding atmosphere. Under conditions of lowered partial pressure of oxygen, compensatory changes in a number of functions (respiration, circulation, composition of blood etc.) occur which are designed to maintain the vital processes of the organism.

The role of interoceptive zones has, however, been inadequately investigated with reference to the dynamics of oxygen saturation of the blood.

W. Grant [2] determined oxygen saturation of the blood in rabbits with denervated carotid sinus and found that inhalation of a mixture containing 10% oxygen led to a drop in the oxygen saturation of arterial blood down to 41% whereas in control animals this value remained within the range of 78-89%. These data were not confirmed by M. Terzioglu, E. Goral and J. Bardav [3].

The aim of the present investigation was to discover more precisely the role of the carotid sinus zones in the dynamics of oxygen saturation of arterial blood under conditions of reduced pressure. The technique of continuous oxyhemometry as developed by Professor E. M. Kreps [1] was employed in this work.

EXPERIMENTAL

The investigation was carried out on cats and rabbits; these were "elevated" in a decompression chamber at the rate of 15 m/sec and exposed for 15-20 minutes to an "altitude" of 7500 m. The rate of "descent" varied within the range 10-15 milliseconds.

The subjects for the experimental series consisted healthy, unoperated animals—4 rabbits and 3 cats, and 5 rabbits and 3 cats which had been subjected to denervation of the carotid sinus zones $2\frac{1}{2}$ months previously. Altogether 47 observations were made.

The animal was attached to a stand with its back up, the head was placed in a holding apparatus. The "saturation measuring device" was attached to the pinna which had been carefully shaved and washed.

The experimenter was placed in the decompression chamber with the animal and took readings every

minute of the relative indications as shown by the apparatus 0-26 (upper scale). The device was set at 100% saturation before the experiment when the animal was inhaling pure oxygen for 2-3 minutes supplied by way of a special mask.

In order to convert the relative readings into percentage saturation a direct Van Slyke calibration was made on 8 analyses on 4 rabbits and 10 analyses on 4 cats. Conversion graphs for the relative readings into percentage saturation are given in Fig. 1, a for cats and 1, b for rabbits.

RESULTS

One series of experiments was carried out on healthy, unoperated animals. The degree of oxygen saturation of arterial blood prior to the "elevations" remained within the limit of 93% for cats and 91% for rabbits. As the atmosphere became more rarefied a more or less regular lowering of oxygen saturation of the blood was noted. On reaching decompression corresponding to 7500 m (287 mm Hg) the saturation dropped to 57-58%. Nonetheless during such exposure in the majority of experiments some rise of saturation was observed; it reached approximately 70% towards the 15th-20th minute.

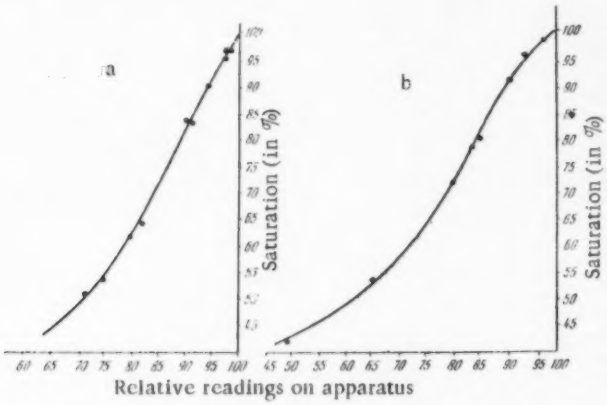

Figure 1. Conversion curve for relative readings into % saturation: a) cats; b) rabbits. Circles indicate results of direct blood gas analyses by the Van Slyke method.

During the increase of pressure to normal the degree of saturation was restored to the original values.

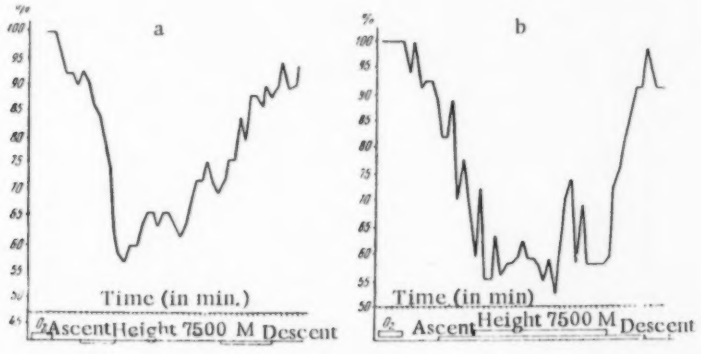

Figure 2. Dynamics of oxygen saturation of arterial blood. a) in intact cat (No. 22) during "elevation" to a "height"; b) in intact rabbit (No. 34). Ordinates – % saturation, abscissae – points from readings taken every minute. Lower line – experimental conditions: initial setting of apparatus at 100% saturation on inhalation of pure oxygen; period of "elevation" to an "altitude"; period of exposure to an "altitude" of 7500 m; period of "descent"; control under normal conditions after the experiment.

TABLE 1

Degree of Oxygen Saturation of Arterial Blood on "Elevation" to 7500 m in Intact Animals (5 minute intervals).

No. of animal	Oxygen saturation of blood prior to elevation		At an elevation of 7500 m												After descent	
			1st minute		5th minute		10th minute		15th minute		20th minute		25th minute			
	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale
Cats																
3	94	96	58	78	60	79	68	83	62	80	64	81	66	82	94	96
22	92	95	60	79	66	82	72	85	72	85	—	—	—	—	94	96
28	94	96	56	76	58	78	81	64	72	85	—	—	—	—	94	96
Average	93.3	—	57	—	61.3	—	73.6	—	69	—	—	—	—	—	94	—
Rabbits																
34	89	92	59	75	56	73	59	75	59	75	69	81	59	75	91	93
36	94	95	55	72	89	95	82	88	72	83	62	77	63	78	94	95
45	91	93	59	75	72	83	80	87	72	83	—	—	—	—	91	93
58	92	94	62	77	55	72	63	78	70	82	—	—	—	—	92	94
Average	91.5	—	58.7	—	68	—	71	—	68	—	65.5	—	61	—	92	—

TABLE 2

Degree of Oxygen Saturation of Arterial Blood on "Elevation" to 7500 m in Animals with Denervated Carotid Sinus Zones (5 minute intervals).

No. of animal	Oxygen saturation of blood prior to elevation		At an elevation of 7500 m												After descent	
			1st minute		5th minute		10th minute		15th minute		20th minute		25th minute			
	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale	%	Readings on apparatus scale
Cats																
16	90	94	55	75	57	77	53	74	49	71	52	73	—	—	78	88
17	92	95	48	70	48	70	48	70	48	70	52	73	—	—	84	91
22	90	94	56	82	52	73	51	72	52	73	—	—	—	—	86	92
Average	90.6	—	53	—	52 3	—	50.6	—	49.6	—	52	—	—	—	82.6	—
Rabbits																
3	76	85	45	62	44,5	61	43	59	44,5	61	49	66	—	—	67	80
6	83	72	38,5	53	41,5	57	44	60	41,5	57	41	56	—	—	67	80
16	78	86	47	64	41,5	57	38,5	52	39	53	38,5	52	39,5	54	72	83
20	80	87	51	68	42	58	40	55	45,5	62	38,5	52	45	61	65	79
28	80	87	40	55	39	53	41	56	42	58	40	55	—	—	67	80
Average	79.4	—	44.3	—	42.1	—	42.1	—	43.1	—	41.4	—	42.2	—	67.6	—

The results of experiments (mean data from repeated experiments) are presented in Table 1.

Table 1 shows both the direct readings on the scale of the apparatus and the percentage saturation determined by the use of conversion curves (see Fig. 1, a and b).

As can be seen from Fig. 2, a, "elevation" was accompanied by a regular drop in the oxygen saturation of the blood.

Having reached 57% during the first minute of being at an "altitude" the oxygen saturation rose to 60% after 3 minutes, and at the 5th minute was already 68%. After a brief drop to 62% it then again showed an increase and reached 72% at the 15th minute. The return to normal pressure was accompanied by a growing increase of saturation.

Figure 2 shows an example of the lowering of oxygen saturation of arterial blood in a rabbit. In this experiment a stepwise drop of saturation was observed during "elevation" to an "altitude" and certain fluctuations during the stay at the "altitude". These phenomena were determined by the frequent vigorous movements of the animal during that time. At the same time in this case too an increase of saturation from 55 to 58% was observed after being at the "altitude" for 15 minutes.

On returning to ordinary conditions the saturation reached the original values.

These experiments showed that in healthy, unoperated animals there was a decrease of saturation of arterial blood with oxygen when the atmospheric oxygen content was lowered; the dynamics of this decrease varied from animal to animal.

The next series of experiments was carried out on animals subjected to preliminary denervation of the carotid sinus zones. The degree of oxygen saturation of arterial blood prior to "elevation" was maintained at 90% in cats and 80% in rabbits.

Considerable diminution of saturation developed during the "elevation" and during the whole period of exposure to conditions corresponding to the experimental "altitude"; the values reached were 51% in cats and 41% in rabbits. During the return to ordinary conditions there was a gradual rise in the saturation as the atmospheric pressure approached normal.

At the same time in the case of all the operated animals the return to ground level conditions was not accompanied by restoration of the initial values of saturation; these remained lower, fluctuating between 78-86% in cats and 64-72% in rabbits during the 7-10 minutes of observation (Table 2).

It should be noted that changes in the degree of saturation may sometimes depend on the individual peculiarities of the experimental animals.

Examples of individual observations are given in Fig. 3, a and b. These show that an operated animal (cat No. 22) exhibited a drop of saturation to 51% which was maintained, approximately, in the course of the whole exposure to "altitude". During the "descent" the saturation showed a gradual increase, but during 5 minutes' observation following the "descent" (under normal conditions) it had not reached the original value, remaining at the level of 82-86% (Fig. 3, a).

A more rapid drop in saturation was observed in the case of rabbit No. 28 (Fig. 3, b): the saturation dropped from the original value of 80% to 41% as early as the first minute on reaching the "altitude" and remained at that level without appreciable deviations during all the time that the animal was exposed to those conditions. During the "descent" the saturation increased gradually but only reached 67% on return to normal atmospheric pressure (at the 7th minute of observation).

The results obtained provide evidence of more profound hypoxemia under conditions of reduced partial pressure of oxygen in animals with denervated carotid sinus zones than in healthy, unoperated animals. It is also characteristic that whereas in the former case the saturation remained almost stationary during the whole period of exposure to "altitude" conditions, in the latter case it exhibited a tendency to growing increase.

There is no doubt that oxygen starvation elicits compensatory mechanisms in the healthy animal which diminish the effect of factors unfavorable for the organism. Deprivation of the organism of interoceptive stimuli from the powerful reflexogenic carotid sinus zones causes marked disturbance of these compensatory mechanisms and consequently decreases the ability of the animal to counteract to some extent the oxygen

lack in the surrounding atmosphere. The delay in restoration of oxygen saturation of the blood upon return to ordinary conditions also indicates disturbance of normal compensatory reactions.

Taking into account the role of the carotid sinus zones in regulation of respiration it may be supposed that the more profound drop of saturation as well as the absence of compensatory saturation under "altitude" conditions observed in animals with denervated carotid sinus zones depend chiefly on disturbances of respiration.

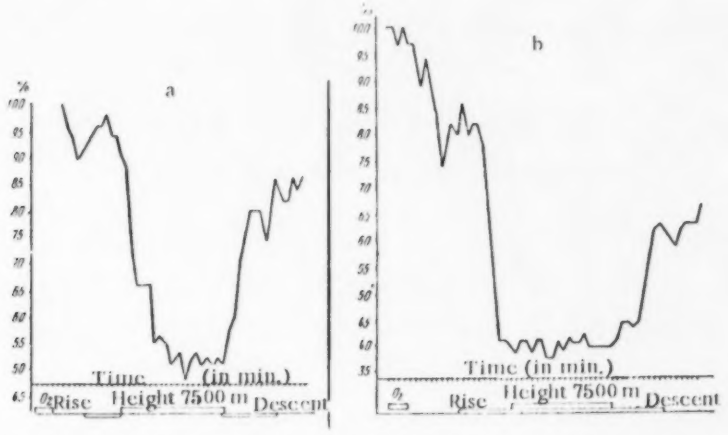

Figure 3. Dynamics of oxygen saturation of arterial blood. a) in cat (No. 22) with preliminary denervation of carotid sinus zones, b) in rabbit (No. 28). Records as in Fig. 2.

The degree of oxygen saturation of arterial blood at reduced partial pressures of O_2 in the inspired air depends to a considerable extent on the normal functioning of the carotid sinus reflexogenic zones which ensure compensatory reactions to oxygen starvation.

SUMMARY

Studies were made of the oxygen saturation in arterial blood in animals during exposure to 7500 m in a chamber. The study was carried out after the photoelectrical method by means of continous oxyhemometry in rabbits and cats with denervation of carotid sinus zones, as well as in controls. In the controls the saturation diminished to 55-62% and increased somewhat after 8-10 minutes' exposition to "altitude". In the experimental animals the diminution of oxygen saturation was more pronounced, reaching 40-50% and remained within this range during the whole period of exposure.

The experiments demonstrated that the dynamics and the degree of oxygen saturation of arterial blood in case of hypoxia depend on the function of the carotid sinus zones.

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THE ROLE OF THE CEREBRAL CORTEX AND SUBCORTICAL CENTERS IN THE REGULATION OF BLOOD CHOLESTEROL LEVELS

COMMUNICATION I: CHANGES IN BLOOD CHOLESTEROL LEVEL ON FORMATION OF A CONDITIONED REFLEX TO RESPIRATORY INHIBITION IN RABBITS

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Disorders of lipid metabolism and particularly that of cholesterol play an important role in a number of pathologic processes, such as atherosclerosis of blood vessels, lipid nephrosis, cutaneous xanthomatosis and others [1, 7, 8].

The work of S. S. Khalatov [7, 8], A. A. Znachkova [3], P. D. Gorizontov [2], N. T. Shutova [9] et al has shown that there is increased diminution of cerebral cholesterol and a rise in blood cholesterol level on emotional excitation, oxygen starvation, intoxication and destructive processes in the brain. On the basis of these facts S. S. Khalatov concluded that a number of hypercholesterinemic conditions were of cerebral etiology.

It was later shown that prolonged narcotic inhibition led to a decrease in blood serum cholesterol and its increase in the brain [4, 6].

Stimulation of the carotid sinus by bacterial filtrate of enteric bacillus [5] and electric stimulation of the brain [10, 11] led to a rise in blood cholesterol.

In the present work investigation of the blood cholesterol levels (using the Engelhardt-Smirnova micro-method) during changes in the functional state of the cerebral cortex and subcortical structures was undertaken.

EXPERIMENTAL

2 series of experiments were carried out on 10 rabbits. Following thrice-repeated estimation of blood serum cholesterol in 4 rabbits its level was determined on formation of a conditioned reflex to defensive reflex inhibition of respiration. The intervals between determinations in the course of the whole investigation did not exceed 5 days.

The sound of a metronome served as the conditioned stimulus. Unconditioned inhibition of respiration was produced by stimulation of the nasal mucosa by threshold doses of CO_2 admitted under a certain pressure onto a reflector held by means of a mask near the animal's nose. The distance between the reflector and the nose was 5 mm. The mask and reflector did not cover the animal's eyes, nose or ears and did not restrict its movements.

The sound of the metronome preceded the action of CO_2 by 3-4 seconds. 6 combinations of the stimuli with 5 minute intervals were used during the experiment; the pneumogram, the application of stimuli and the time were recorded on a kymograph (Fig. 1, C).

• Communication presented at the scientific conference of the Pediatric Medical Institute in 1956.

Formation of the conditioned reflex passed through the stage of conditioned reflex increase in the rate of respiration and decrease in the amplitude of respiratory movements (Fig. 1, B). The typical form (cessation of respiration) of the conditioned reflex (Fig. 1, D) was established in rabbits (No. 2 and 4). In two other rabbits (No. 3 and 1) the reflex in most cases manifested itself by increased rate and decreased amplitude of respiration (Fig. 1, B) and only in 5-7% by cessation of respiration.

The first series included 217 experiments using a conditioned stimulus and 168 blood serum cholesterol estimations. The animals were kept under observation for 6 months.

RESULTS

During the formation of the conditioned reflex, as early as 10-15 days after the beginning of the experiments (60-80 combinations) all the rabbits showed a 25-30% decrease of blood serum cholesterol. On further prolonged work with the animals the average content of cholesterol decreased by 50% (Table 1) as compared with the average initial level. The maximal decrease noted towards the end of the procedure amounted to 80% in some cases.

A comparison of the number of positive responses to the conditioned stimulus (increase in respiratory rate with decrease in amplitude, cessation of respiration) and the degree of decrease of blood serum cholesterol shows that in the case of rabbit No. 3 the conditioned reaction appeared in a smaller percentage of cases than in other rabbits and the decrease in blood serum cholesterol in the same rabbit was insignificant (Table 2).

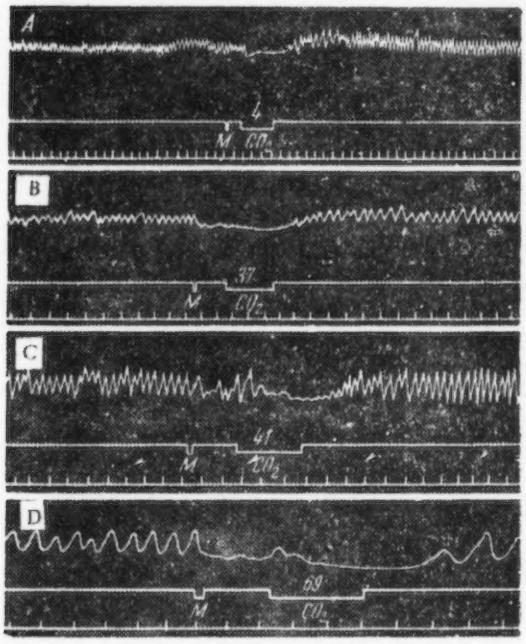

Figure 1. Respiration in rabbit during various periods of the formation of conditioned reflex. Records (from above down): respiration, beginning of stimulation, time marker in seconds; the figures refer to the serial number of combination of stimuli. M – metronome in A, B, C, D.

Therefore the decrease in cholesterol content is in direct ratio to the extent to which the conditioned reflex is pronounced.

On prolonged and continuous work with the animals disturbances of conditioned reflex activity were observed, which manifested themselves in a decrease of positive responses to the conditioned stimulus. These disturbances as a rule corresponded with minimal blood serum cholesterol levels. Towards the end of a two-months interval following termination of experiments using a conditioned stimulus the blood serum cholesterol remained decreased in 2 rabbits; in two others the cholesterol level exceeded the original value by 60-75%.

In the second series of experiments the blood serum cholesterol levels were studied in rabbits under conditions of dietary cholesterol "loading" against a background of conditioned reflex activity.

Observations were carried out successively on 3 groups of animals. In each group there were 2 experimental and 2 control rabbits which received cholesterol in equal amounts. In the first and second groups, experiments using a conditioned stimulus were begun on the experimental animals after 4-6 feeds of cholesterol when its level had risen 3-4-fold as compared with the initial content.

First group (Table 3). Experimental rabbits No. 1 and 4 and control rabbits No. 5 and 6 were given 0.5 g crystalline cholesterol in 40 ml milk. The increment of cholesterol in the blood serum towards the end of feeding found in the experimental rabbits was 5 and 8 mg % respectively. In the course of the same period of feeding the cholesterol increment in control rabbits was 164 and 166 mg %.

TABLE 1

Changes in Cholesterol Levels in Rabbits During Formation of Conditioned Reflexes

No. of rabbit	Blood serum cholesterol							Number of experiments using conditioned stimulus
	Average of 3 determinations	During conditioned reflex activity				After 60 days		
		Average of 17 determinations		Minimal in mg %	Minimal in % of initial	Average of 3 determinations		
	Initial mg %	In mg %	In % of Initial			In mg %	In % of Initial	
1	37	21	56	10	27	22	59	57
2	25	19	76	10	40	44	176	54
3	50	33	66	20	40	80	160	52
4	24	13	54	5	20	14	58	54

TABLE 2

Manifestation of Conditioned Reflex in % of Total Number of Stimulations

No. of rabbits	Respiratory changes	Cessation of respiration	Presence of conditioned reaction	Absence of response
1	77.8	5.5	83.3	16.7
2	36	60	96	4
3	70.6	4.1	74.7	25.3
4	40.6	54.7	95.3	4.7

The average blood serum cholesterol content in rabbits No. 1 and 4 during feeding was 62 and 32 mg%, the average content in control rabbits No. 5 and 6 was 217 and 141 mg%. Details of conditioned reflex activity in the experimental rabbits are given in Table 4.

Second group. Experimental rabbits No. 2 and 3 and control rabbits No. 7 and 8 received 38 doses (0.5 g) of cholesterol over a period of 70 days. By the end of feeding (Table 3) the increment of blood serum cholesterol in experimental rabbits No. 2 and 3 and 85 and 431 mg% respectively. In control rabbits No. 7 and 8 the increment was equal to 246 and 573 mg%. The average blood serum cholesterol content during feeding was 64 and 258 mg% in experimental rabbits. In control animals it was 162 and 393 mg%.

Thus, in 3 out of 4 experimental rabbits (from the first and second groups) ingestion of cholesterol against the background of conditioned reflex activity did not result in hypercholesterinemia, and the cholesterol increment towards the end of feeding was insignificant. In one experimental rabbit (No. 3), as well as in the control rabbits, hypercholesterinemia was clearly evident. It must be remarked that the degree of manifestation of the conditioned reflex was much less in rabbit No. 3 than in the other rabbits (Table 4).

TABLE 3

Blood Serum Cholesterol Content

No. of rabbit	Blood cholesterol content in mg %					Number of cholesterol feeds	Duration of observation (days)
	Initial	At the end of feeding	Increment towards end of feeds	Average (of 11) during feeds	Average increment during feeding		
1	28	36	8	62	34	34	64
4	15	20	5	32	12	34	64
5	39	203	164	217	178	28*	53
6	24	190	166	141	117	28*	53
1_2	14	30	16	75	61	19	36
4_2	20	53	33	77	57	19	36
9	21	206	185	241	220	19	36
10	86	396	310	302	218	13*	26
2	21	106	85	64	43	38	70
3	35	466	431	258	223	38	70
7	40	286	246	162	122	38	70
8	60	633	573	393	333	38	70

TABLE 4

Manifestation of Conditioned Reflex in % of Total Number of Stimulations

No. of rabbit	Respiratory changes	Cessation of respiration	Presence of conditioned reaction	Absence of response	Number of experiments using a conditioned stimulus
1	92	3	95	5	18
4	34	66	100	0	19
1_2	68.0	9.3	77.3	22.7	24
4_2	68.0	29.3	97.3	0.3	24
2	56	40	96	4	28
3	76.1	0.7	76.8	23.2	27

Following a three-months interval experiments were repeated on rabbits No. 1 and 4. A new pair of control rabbits (No. 9 and 10) was taken, and the animals were fed egg yolk. Four egg yolks were mixed with milk and the volume made up to 160 ml. A single feed consisted of 40 ml of mixture of milk with one egg yolk (0.3 g cholesterol). Each rabbit received 19 yolks in the course of 36 days. Results of the experiments are presented in Tables 3 and 4. By the end of feeding the blood cholesterol increment in the experimental rabbits No. 1_2 and 4_2 was 16 and 33 mg%. During the same period the control rabbit No. 9 showed a cholesterol increment of 185 mg% and rabbit No. 10 showed an increment of 310 mg% over a considerably shorter period (13 feeds). The average blood serum cholesterol content in experimental rabbits No. 1_2 and 4_2 during feeding was 75 and 77 mg%; the average cholesterol content in control rabbits No. 9 and 10 was equal to 241 and 302 mg%. It is thus seen that ingestion of egg yolks against the background of conditioned reflex activity did not lead to the development of hypercholesterinemia in rabbits. No changes in the conditioned reflex activity of rabbits could be observed during administration of cholesterol. A more or less constant level of such activity was maintained.

In summary, the results of the present series of experiments indicate that during conditioned reflex activity the blood serum cholesterol increment was insignificant in 5 out of 6 cases by the end of the feeding and there was no development of hypercholesterinemia in rabbits (Fig. 2). During the same period in 8-10-fold rise in blood serum cholesterol was observed in control rabbits (Fig. 2). It must also be noted that the level of blood serum cholesterol during feeding was in inverse relation to the extent of conditioned reflex manifestation.

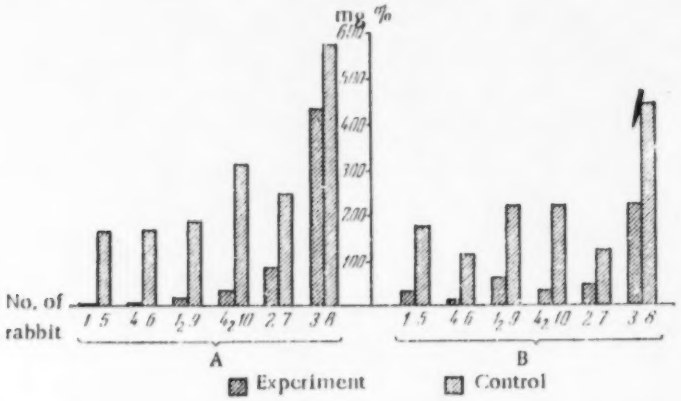

Figure 2. Cholesterol increment in blood serum of rabbits during ingestion of cholesterol. A) Increment towards end of feeding (in mg%); B) average increment during feeding (in mg%).

Thus, rabbit No. 3 showed development of hypercholesterinemia on feeding, and exhibited a weak type of nervous system with the lowest number of positive responses to the conditioned stimulus.

Upon cessation of observations the experimental and control rabbits were sacrificed and a histologic examination of the blood vessels with respect to lipids was carried out. It was found that both the experimental and control animals showed primary lipoidosis of the vessels despite the low blood serum cholesterol in the former group. The data obtained, with their evidence of primary deposition of lipids within the blood vessel wall in rabbits, pose the question whether it is possible for vascular lipoidosis to develop at normal blood cholesterol levels in the presence of altered nervous regulation.

SUMMARY

Development of a conditioned reflex to inhibition of respiration in the rabbit is accompanied by a decline of blood cholesterol. Feeding cholesterol to animals in a state of conditioned reflex activity did not result in hypercholesterinemia, while in control animals cholesterol feeding caused a rise of blood serum cholesterol. The cerebral cortex and subcortical formations are thus participating in the regulation of cholesterol metabolism.

Preliminary data demonstrating deposition of lipoids in vascular walls of experimental rabbits, point to the possibility of vascular lipoidosis being caused by altered nervous regulation, the cholesterol blood level being normal.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

CHANGES IN THE EXCITABILITY OF THE VASOMOTOR CENTER DURING SURGICAL OPERATIONS ON THE AURICLE

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During operations on patients with stenosis of the left atrioventricular foramen there are recorded definite changes in pulse, electrocardiogram, and vascular tone, which arise at the moment the auricle is manipulated.

V. I. Proznina [3], B. S. Kulaev [1], and others showed that irritation of the pericardium is attended by considerable changes in blood pressure and vascular reflexes, i.e., changes in the vasomotor center.

The present work was devoted to the study of the excitability of the vasomotor center during surgical operations on the auricle.

METHODS

The excitability of the vasomotor center was investigated by stimulating the baroreceptors of the carotid sinus reflexogenic zone. The experiments were carried out on rabbits. Twenty-four experiments in all were carried out.

Under urethane anesthesia, we secured the animals to the bench on their backs. We isolated the carotid arteries, one of which we arranged for blood pressure recording via mercury manometer and to obtain the pressor reflexes of the carotid sinus every 5-10 minutes, we put a Dieffenbach clamp on the other carotid. The pressure on the common carotid lasted 20 seconds. We determined the initial magnitude of the carotid sinus pressor reflex before the start of the operation.

Operation procedure: In the operation we exposed the ribs and sternum with a long skin-muscle incision. From the second to third ribs we cut the costal cartilages and removed part of the sternum. By blunt dissection we exposed the pericardium and made a medium sized incision in it; through the opening we grasped the auricle and drew it through the incision. Then we applied a ligature to the auricle as a preliminary to suturing it.

The ligatured auricle retreated into the incision, which we sewed up tightly.

We recorded the carotid sinus reflex after cutting into the costal cartilages and removing the sternum, after cutting into the pericardium, after grasping and suturing the auricle, and during the 2-3 hours after the operation.

RESULTS

In all the experiments, immediately after the ligation of the auricle, there was a decrease in the pressor reflex, up to the point of its complete disappearance at the end of the first or the beginning of the second hour. The latent period of the reflex was notably prolonged.

The blood pressure level remained the same or fell a very little. In some experiments, when the common carotid was squeezed, inverted (depressor) reflexes were observed (Fig. 1).

Thus ligature or suturing of the auricle is reflected in the excitability of the vasomotor center. Definite diminution of, or inversion of, pressor reflexes, observed after the operation, testifies to a lowering of the excitability of the vasomotor center.

To investigate the mechanism of the effect observed, a number of experiments were carried out.

In the first series, before the experiment or soon after it, we cut the vagus nerves, or blocked them with novocaine.

In all 4 experiments neither section nor blocking of the vagus nerves exhibited any influence on the dynamics of the change in reflexes following the operation.

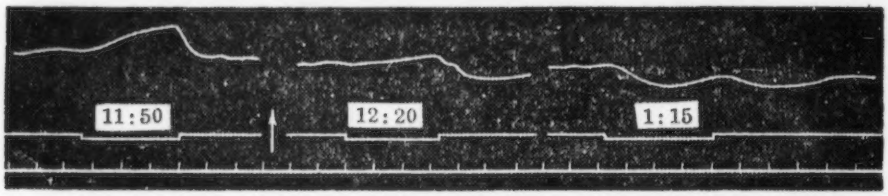

Fig. 1. Change in the pressor reflex of the carotid sinus following ligature of the auricle. Meaning of curves (from top to bottom): blood pressure, records of stimuli, time signals (5 second intervals), ↑ -ligature of auricle.

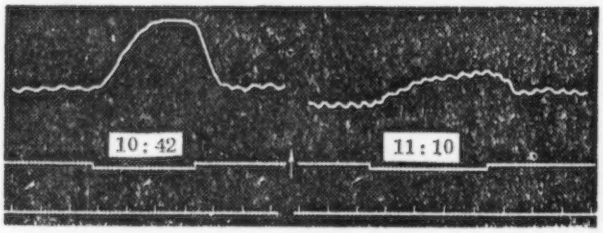

Fig. 2. Changes in the carotid sinus pressor reflex on suturing the auricle after section of the vagus nerves. Meaning of curves same as in Fig. 1.

In rabbits with cut vagi, ligature of the auricle led to lowering of the excitability of the vasomotor center, which was expressed as a decrease, disappearance, or inversion of the carotid sinus reflexes (Fig. 2).

The morphological investigations of B. M. Erez [4], E. K. Plechkova [2] and others demonstrated the role of depressor fibers in the innervation of various regions of the heart.

In this connection we carried out the following experiments, in which we cut the depressor nerves about 1-1 ½ hours after operation on the auricle, i.e., when the reflexes were already sharply decreased.

In 4 experiments, immediately after section of the depressor nerves, the carotid sinus reflexes were completely restored. In one experiment the magnitude of the reflexes after section of the depressor nerves was increased, reaching values higher than the initial values.

In the subsequent 7 experiments we performed the ligature of the auricle on animals whose depressor nerves

had already been cut. Section of the depressor nerves before the operation had little effect on the magnitude and character of the carotid sinus pressor reflexes; in the majority of cases the reflex level was not changed by section of these nerves. In all cases, ligation of the auricle, in animals whose depressor nerves had already been cut, was not reflected in the state of the carotid sinus reflexes; their magnitude and period of inhibition during the first 2 hours after operation remained as before (Fig. 3), and only at the end of the 2nd or the beginning of the 3rd hour, along with a gradual fall in blood pressure in two experiments, did the carotid sinus reflexes decrease.

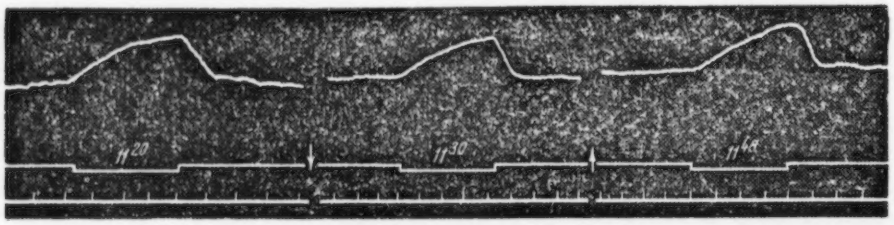

Fig. 3. Carotid sinus pressor reflex before and after ligature of the auricle on a background of a section of depressor nerves. Meaning of curve as Fig. 1. ↓ section of depressor nerves; ↑ ligature of auricle.

Consistency of the carotid sinus reflexes was observed only if both depressor nerves were cut. After section of only one depressor nerve, suture of the auricle led to a decrease in the reflexes, as before.

Thus, section of the depressor nerves completely eliminated the change in the state of excitability of the vasomotor center which was observed when the auricle was operated on. This fact allows us to suppose that in operative manipulation of the auricle, afferent impulses reach the vasomotor center, through the depressor nerves leading to a diminution of its excitability, which may be one of the reasons for disturbances in hemodynamics during operations on the heart.

SUMMARY

The excitability of the vasomotor center was investigated by stimulating the baroreceptors of the carotid sinus zones of 24 rabbits, anesthetized with urethane, during operations on their auricles. Ligation of the auricle caused a decrease in the excitability of the vasomotor center, which was expressed as inhibition, which could even be complete, of the pressor reflexes. Section of both vagi did not abolish this effect. Section of both depressor nerves completely abolished these reactions.

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DYNAMICS OF HEMATOLOGIC INDICATORS DURING ACUTE EXPERIMENTAL BLOOD LOSS

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(Presented by Active Member of the USSR Acad. Med. Sci. Prof. M. D. Tushinsky

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The aim of our work was elucidation of the reasons for the different reactions of sick persons to acute blood loss.

METHODS AND RESULTS

The experiments were carried out on female rabbits. Six series of experiments, 70 experiments in all, were set up. During the examination of the blood we took into account the difference in hemoglobin percent, if it was not less than 5, in estimating the erythrocyte count (over 200,000) and leukocyte count (over 400 formed elements).

In the first preliminary series of experiments we investigated whether there is a difference in the blood picture of blood taken from normal animals, in the vessels of the internal organs and the ear, and how laparotomy affects the composition of the blood.

Ten experiments were set up. Before the operation we took blood from the animal's ear for investigation of the percent hemoglobin and the red and white counts.

We carried out the laparotomies under local anesthesia (0.25% novocaine solution), after which we took blood simultaneously from the ear, the uterus, and the mesentery of the small intestine. Laparotomy did not produce any change in the condition of the blood. We examined all the blood taken for percent hemoglobin and numbers of erythrocytes and leukocytes.

The distribution of the formed elements of the blood in the vessels of the internal organs and the peripheral portions of the body proved to be the same. Differences were within the limits of possible error and had no practical significance.

Laparotomy did not produce any change in the composition of the blood.

In the second series of experiments we investigated the amount of blood loss for fatal hemorrhage in rabbits.

We carried out experiments on 6 female rabbits. We defined the total amount of blood as 1/20 of the animal's weight. Then we isolated the carotid artery and from an incision in it we allowed a thin stream of blood to run out through a cannula into a graduated cylinder until such time as the rabbit died.

For female rabbits of average weight a blood loss of 53.5% of the total mass of blood proved fatal, which confirms the data of D. O. Ott [2] and Sh. M. Leites [1].

The object of the third series of experiments was to investigate the blood picture in the peripheral vessels

and the vessels of the internal organs after acute hemorrhage.

Nineteen experiments were set up. After a preliminary analysis of the peripheral blood we conducted a blood letting from the carotid artery of 30% of the total mass of blood.

We allowed the necessary quantity of blood to run through a cannula into a graduated cylinder, after which we tied the carotid with a catgut ligature. About half an hour after the blood letting we carried out, under local anesthesia, a laparotomy and investigated blood from the peripheral blood vessels of the ear and at the same time from the vessels of internal organs – uterus and the mesentery of the small intestine.

In all 19 of the rabbits, after acute hemorrhage the percent of hemoglobin and the number of erythrocytes decreased, both in the peripheral vessels and in the vessels of the internal organs. However the decrease in percent hemoglobin and erythrocyte count, in 15 out of 19 experiments, proceeded unequally in different local circulatory channels. In 10 rabbits the greatest decrease in hemoglobin percent and erythrocyte count was observed in blood from the vessels of the ear. In the vessels of the uterus and the mesentery of the small intestine, the blood, after acute hemorrhage, contained more hemoglobin and erythrocytes.

All 10 rabbits stood the blood letting and laparotomy well and their condition soon became normal.

In 5 rabbits the opposite relation of the blood indicators was observed: the hemoglobin percent and red count in the ear vessels was higher than in the vessels of the internal organs. All rabbits with similar blood differences died in a few hours immediately after the experiment.

In 4 rabbits the hemoglobin percent and the number of erythrocytes were the same in all parts of the circulation. Two of these died soon after the experiment, but 2 rabbits recovered.

In 14 hour of 19 experiments the number of leukocytes in the ear vessels after acute hemorrhage was higher than in the vessels of the internal organs, and even occasinally surpassed the prehemorrhage figures.

Thus the decrease in the indicators of the blood following hemorrhage proceeded unequally in different parts of the circulation. Those animals in which the blood of the internal organs was richer in hemoglobin and erythrocytes than the periphery bore the experiment well. Those rabbits, in which investigation of the blood showed a higher hemoglobin and erythrocyte content in the peripheral regions of the body died. In a moderate numbers of cases (4 experiments), uneven distribution of morphological elements of the blood after hemorrhage was not observed.

Leukocytosis after hemorrhage appeared essentially only peripherally.

In the fourth and fifth series of experiments we investigated the causes of similar unequal distributions.

In the fourth series 10 experiments were conducted. Under general ether anesthesia we allowed blood to be lost to the amount of 30% of the total blood and about half an hour later performed a laparotomy. General anesthesia was employed to exclude the influence of the central nervous system.

After blood loss under ether anesthesia the hemoglobin and erythrocyte levels were the same in all parts of the circulation. After hemorrhage 8 rabbits died and only 2 survived it comparatively well.

It is evident that the unfavorable outcome of the loss of blood depended on the exclusion of the central nervous system. The rabbits did not tolerate the same loss of blood as the animals in the third series of experiments under the usual conditions. The number of leukocytes was the same in all parts of the circulation.

In the fifth series (15 experiments) we performed the blood letting about half an hour after the intravenous injection of benzedrine, which we introduced in the amount of 0.6 mg/kg, purposing to stimulate the central nervous system. About half an hour after the blood letting we performed a laparotomy under local novocaine anesthesia.

Under these conditions in 13 of 15 rabbits the blood after hemorrhage was significantly richer in hemoglobin and erythrocytes in the internal organs than the blood of the peripheral vessels of the ear. All 13 rabbits tolerated the experiment well.

In 2 rabbits after blood letting there was observed in the peripheral vessels a higher level of hemoglobin and erythrocytes than in the internal organs. Both rabbits died immediately after the experiment. Investigations

of the white cells in the blood of the animals of this series after blood loss showed the posthemorrhagic leukocytosis established by us earlier.

In the sixth series of experiments we investigated the morphological composition of the blood in the vessels of the cerebrum after acute hemorrhage.

Ten experiments were set up. About half an hour after the blood letting from the carotid we performed a laparotomy and immediately took blood from the ear, uterus, mesentery, small intestine and from the cerebral vessels (branches of the middle cerebral artery).

In 8 out of 10 rabbits the blood from the cerebral vessels, uterus and mesentery contained more hemoglobin and erythrocytes than blood from the peripheral vessels. In 1 experiment there was the reverse relationship, i.e., the peripheral blood was richer in hemoglobin and erythrocytes than the blood from the brain and other internal organs.

In 1 rabbit we did not establish any difference between blood from differentparts of the circulation.

Leukocytosis after blood loss was also on the whole peripheral.

Thus rabbits bore acute hemorrhage differently. To a certain extent this depends on the conditions of the experiments. Those animals stand acute hemorrhage best, in the internal organs of which the blood after hemorrhage is richer in hemoglobin and erythrocytes than in the peripheral vessels of the body. Evidently this is a protective reaction of the body which may be called a compensatory redistribution of the morphological elements of the blood. In the establishment of this the central nervous system plays a big role. In those cases where there was a reverse redistribution of the blood and the peripheral network of vessels proved to better supplied with blood, all the animals succumbed. Such a redistribution of the morphological elements of the blood following blood loss we may call pathological.

SUMMARY

Experiments on female rabbits showed that those of them survive acute blood loss whose blood in the internal organs and cerebral vessels is richer in hemoglobin and erythrocytes than is the blood of the peripheral vessels. Anesthesia diminishes these differences and reduces the number of animals surviving. The reverse effect is produced by benzedrine.

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TREATMENT OF EXPERIMENTAL NEUROSES IN THE DOG BY MEDICAMENTOUS SLEEP

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(Received October 4, 1956. Presented by Active Member of the Academy of Medical Sciences Prof. V. N. Chernigovsky)

Sleep therapy is widely applicable to the treatment of neuroses and somatic illnesses which originate from functional disturbance of the cortical activity of the cerebral hemispheres (ulcers, hypertension, etc.). In the treatment of these diseases clinicians do not always obtain positive results. For this reason there is an urgent need to work out experimental sleep therapy of neuroses in suitable animals.

In this connection the experiments of M. K. Petrova are of particular interest [1, 2], where veronal sleep therapy was carried out on dogs weakened by castration, prolonged experiments, various diseases, and dogs with senile changes in the function of the central nervous system. In all cases M. K. Petrova observed a favorable influence of sleep therapy on dogs which expressed itself by an improvement in their general condition (disappearance of dystrophic changes of the skin, etc.). A protracted restoration of conditioned reflex activity to normal was achieved in only one dog. In the other animals there was observed only an insignificant and transient improvement of conditioned reflex activity.

Our experimental work was devoted to the investigation of the questions: what disturbances of higher nervous activity yield the most improvement or normalization under the action of sleep therapy, what regime of sleep therapy shows the most favorable influence on the restoration of cortical processes, what soporific, in what combinations and doses proves to be best for the treatment of experimental neuroses.

METHODS

We carried out experiments on 5 dogs; Alpha, Fiston, Don, Orl, and Uranus. in all dogs there were produced conditioned food reflexes by the (classical) Pavlov method. A positive conditioned reflex was produced in all dogs to the sound of a metronome with a frequency of 120 beats per minute and to the light of an electric bulb of 100 candle power, the differentiated stimulus was the sound of a metronome with a frequency of 60 beats per minute. We employed the conditioning stimuli for each dog always in one and the same order, at intervals of 5 minutes. During the experiment we recorded the secretion of the salivary gland, feeding reaction, and frequency of respiration. The type of higher nervous activity was determined by the customary small standard of assay, with some exceptions (in the dogs Don, Orl and Uranus the establishment of a system of conditioned reflexes and a series of assays of the type of higher nervous activity was carried out by our scientific coworker I. A. Zachinyaeva).

Neuroses were produced by influencing the weakest side of the higher nervous function of the experimental animals. In the experiments we made use of an increase in the time of action of the conditioning stimulus, as in the course of an experimental day, or in the course of several days, weeks, or months. In dogs of a strong type, neurosis appeared as a simultaneous stress of the basic nervous processes and their mobility. For this we

used in the course of one experiment an extra loud rattle, sometimes between the conditioning stimuli, or sometimes during their action simultaneously with a sharp increase in the time of the isolated action of the positive conditioning stimulus to 50 seconds, but the differentiated stimulus to 2 minutes.

After the production of disturbances of higher nervous function in the animals, we carried out, in the course of several days, observations on the dynamics of the development of these disturbances, then broke off the experiments for 10-14 days, and if the rest did not produce improvement in the animals' condition, carried out therapy by inducing sleep.

We produced the medicamentous sleep, in a special room, for 3 to 6 days in succession. In the course of 3 days the dogs slept from 30 to 52 hours, in the course of 6 days, from 52 to 100 hours. For a soporific we employed barbamyl* and a mixture of barbamyl and chloral hydrate (1 : 3). We gave the barbamyl to the dogs with food, the mixture we introduced into the rectum.

We adjusted the doses of the soporifics, depending on the typological pecularities of the experimental animals, so that the sleep should be close to physiological sleep. In such sleep the dogs retained pain sensitivity, the corneal reflex, and the orientation reaction to a loud noise.

If after the first application of sleep therapy normalization of the higher nervous function of the animals did not ensue, then after 2-9 months we repeated the medicamentous sleep.

RESULTS

In the process of the experiments prolonged disturbances of higher nervous function were produced in dogs, which could be characterized in the terminology of the Pavlov school as neurosis with predominance of inhibition, since it is accompanied by a weakening in the first place of the process of excitation and the development in the cortex of the cerebral hemispheres of the dogs of a limiting protective inhibition.

With the dogs Fiston and Don, belonging accordingly to the weak and intermediate type of higher nervous activity, the neuroses were characterized by disturbances of general gray matter processes, but the unconditioned feeding reflex was not disturbed.

Externally these neuroses manifest themselves by a 12-80% lowering of the amount of conditioned reflexes, the constant occurrence of hypnotic intervals, mainly equalizing and paradoxical, manifestations of explosiveness and rapid exhaustion of the process of stimulation, and an increase of 25-100% in the frequency of respiration during the experiment. Dogs with these neuroses ate all their food, which was given after the action of the conditioning reflexes, and their reaction under experimental conditions was always positive. Prolonged interruption of the experiments (from 7 days to a month) did not favor improvement in the higher nervous function of the dogs with these neuroses.

In these cases, after a three day sleep under the influence of barbamyl, the magnitude of the conditioned reflexes increased 20-75%, the periodic manifestations and the manifestations of sudden exhaustion of the conditioned reflex stimulus disappeared. Normal higher nervous function was observed in these dogs in the course of 4-5 months after the application of sleep therapy.

Prolonged disturbance of higher nervous function, obtained in dogs Alpha, Orl, and Uranus (assigned accordingly to the strong type with some predominance of the excitatory process, to the strong stable, and to the weak type of higher nervous activity), was characterized by disturbances of the course of gray matter processes and a sharp disturbance of the unconditioned feeding reflex. Refusal of food by the animals during the experiment, either in the presence of the experimenter or in his absence, was observed for several months to 2 1/2 years. Apart from this, there was observed chaotic conditioned reflex activity, a considerable decrease or complete disappearance of conditioned secretion, an increase to 8-20 times in respiration during the experiment and the continued presence in the animals of a negative reaction to the conditions of the experiment (they had to be carried to the machine in the arms). Complete starvation for 1-9 days worsened the dog's higher nervous activity. Interruptions of the experiments for 10-50 days did not promote improvement of the condition of the animals. Three day and six day sleep under the influence of barbamyl produced a slight and temporary improvement in the conditioned reflexes for 2-3 days which was expressed as some increases in the magnitude of the conditioned secretion, a decrease in the respiration rate during the experiment, and more active behavior of the animals under the experimental conditions.

* Russian trade name.

We induced sleep by a mixture of soporifics for 3 days. We introduced the soporific barbamyl and chloral hydrate into the rectum at intervals of 15-20 minutes. To dogs of the weak type of higher nervous activity we gave 2 g of this mixture, to dogs of the strong type, 2.6 g.

In neuroses which manifested themselves as disturbances of the conditioned feeding reflexes without expression of disturbances of the unconditioned feeding reflex, sleep under the influence of the mixture of soporifics produced complete and lasting normalization of the higher nervous functions of the experimental animals.

In the case of neuroses accompanied by disturbances of the conditioned feeding reflex and the unconditioned feeding reflex, sleep under the influence of the mixture of soporifics exerted a more favorable action than did sleep under the action of barbamyl alone. After sleep there was observed a definitely improved attitude of the dogs in regard to the experimental conditions. They leaped by themselves to the machine and their unconditioned feeding reflex was restored, i.e., after sleep these dogs ate all the food offered them in the conditioned reflex room. However, their conditioned reflex activity was just as chaotic as before their sleep, and their respiration rate during the experiment was considerably higher than normal.

DISCUSSION

In evaluating the effectiveness of various aspects of medicamentous sleep it appeared that neuroses in dogs, characterized by disturbances of the course of general gray matter processes, completely disappeared after three-day sleep under the influence of barbamyl alone or under the influence of a mixture of soporifics. Neuroses characterized by disturbances of conditioned feeding reflexes could not be completely abolished, in spite of repeated application of sleep therapy, whether under the influence of barbamyl alone or under the influence of a mixture of soporifics. This fact is clearly evidence of the qualitative difference between the limited protective inhibitions of these neuroses and of the necessity of a different approach to the therapy of different aspects of neurosis in which the inhibitory process predominates.

It should also be mentioned that sleep under the influence of a mixture of soporifics has a more beneficial influence than sleep under the influence of barbamyl alone. This is evidently explained by the fact that soporifics introduced together act unequally on different parts of the central nervous system; therefore their employment brings about a simultaneous combined effect on the activity of the cortical and gray matter centers.

The use of a mixture of soporifics had this advantage, that under its influence the sedation of the experimental dogs took as a rule 20-30 minutes, while the time under the influence of barbamyl was $1\frac{1}{2}$ - 2 hours. When the mixture was employed the animals went to sleep quietly, but when barbamyl was used there was usually observed decided active excitement, especially evident in dogs of the weak and intermediate types of higher central nervous activity.

From the above data it is possible to suggest that the employment in the clinic of suitable mixtures of soporifics for sleep treatment of disturbances of the central nervous system might increase the effectiveness of sleep therapy.

SUMMARY

Complete normalization of the higher nervous function of neurotic dogs with disturbed cortical processes predominating was achieved by three-day sleep produced by barbamyl alone or in combination with chloral hydrate. If in addition to the cortical processes the unconditioned feeding reflex was also disturbed, improvement after sleep treatment lasted for only 1-2 days. Sleep induced by a mixture of drugs was more effective than that induced by barbamyl alone.

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PHARMACOLOGY

THE EFFECT OF SOME PHENOTHIAZINE DERVATIVES ON THE DEVELOPMENT OF EXPERIMENTAL TOXIC EDEMA OF THE LUNGS

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There have recently appeared clinical and experimental studies in which attention is drawn to the successful use of derivatives of phenothiazine for therapy of pulmonary edema of various origins.

A. Decortis [5] observed two clinical cases where they treated severe edema of the lungs by the introduction of a lytic mixture proposed by H. Labori and P. Huguenard (chlorpromazine, promethazine, diethazine). There are also similar communications concerning the effectiveness of chlorpromazine, pacatal* [6], and promethazine in the treatment of experimental edema of the lungs.

In the present study we presented ourselves with the problem of comparing the effectiveness of aminazine,* mepazine, its chlorinated derivative, and promethazine in experimental therapy of toxic edema of the lungs.

METHODS AND RESULTS

Mepazine (synonym, pacatal) and its chlorinated derivative were synthesized in the Institute of Pharmacology and Chemotherapy of the USSR Academy of Medical Sciences. We studied the action of each preparation in experimental edema of the lungs produced by adrenalin in white mice, and pulmonary edema resulting from the administration of ammonium chloride to white rats.

In different series the number of animals was different, but sufficient for statistical reliability of the results.

Pulmonary edema produced by adrenalin. The first series of experiments (20 mice) served as a control. We produced adrenalin edema of the lungs by the administration of the official preparation of adrenalin in 0.1% solution in the amount of 10 mg/kg of body weight. About half of the animals of this series succumbed in 8-59 minutes; we killed the others by decapitation an hour after administration of the adrenalin. In all cases we examined the lungs carefully, removed them and weighed them. In addition to the macroscopic picture of edema of the lungs (swelling, blotchiness, hemorrhage, and flow of foamy liquid from incised organ), we determined the weight of the lungs as a percentage of body weight (K). Normally this amounts to 0.7-0.9. In the experiments of the control series it was higher than 1.25, in some cases going as high as 2.03-2.31. The average value of K for the control series was 1.59.

In the second series of experiments (20 mice), about an hour before intraperitoneal injection of the adrenalin we administered aminazine in the amount of 10 mg/kg body weight. At the time the adrenalin was injected the mice were sluggish, immobile, and in a state suggesting drowiness. In 16 cases edema did not develop, in 3 cases the symptoms of edema were slight, in 1 case marked. Average value of K., 0.83.

* Russian trade name.

In the third series of experiments we administered a subcutaneous injection of adrenalin about an hour after administration of mepazine. In a dose of 50 mg/kg (20 mice), in 8 cases mepazine completely prevented the development of edema, in 12 cases there was edema with K = 1.14 (average value) in a dose of 100 mg/kg (12 mice), in 9 cases mepazine prevented the development of edema; in 3 cases edema was observed. Average value of K, 0.99. The preparation, administered in the same dose intraperitoneally (9 mice) in all cases prevented the development of edema; K was 0.75 (average value).

In the fourth series of experiments we studied the action of the chlorinated derivative of mepazine (100 mg/kg). When injected subcutaneously (10 mice), this preparation in 1 case prevented the development of edema, in 4 cases the edema was mild, in 5 marked; average value of K = 1.28. Intraperitoneal administration (8 mice) of the preparation completely prevented the development of edema; K = 0.79 (average value).

In the fifth series of experiments (9 mice) we employed the subcutaneous injection of 50 mg per kg of promethazine to prevent edema. In 3 cases edema did not develop, in two it was mild, in 4 cases, marked; K = 1.24 (average value).

Pulmonary edema produced by ammonium chloride. We injected ammonium chloride intraperitoneally in the amount of 0.70-0.75 ml of a 6% solution per 100 g body weight. In the first (control) series of experiments (31 rats), in the majority of cases (one exception) the symptoms of edema were strongly pronounced. The magnitude of K ranged from 0.68 to 2.21 with an average value of 1.47.

In the second series of experiments, about an hour before the administration of the ammonium chloride we injected 10-20 mg/kg of aminazine subcutaneously. In a dose of 10 mg/kg (20 rats) aminazine in 15 cases completely prevented the development of edema, in 3 cases edema was slight, and in only two pronounced; the average value of K was 0.89. Administration of 20 mg/kg of aminazine (10 rats) averted toxic edema in 9 cases; average value of K = 0.74.

In the third series of experiments we studied the action of 50 and 100 mg/kg of mepazine when injected subcutaneously. Following the injection of 50 mg/kg (11 rats), edema did not develop in 4 cases, was mild in 5 cases; K = 1.04 (average value). Following the injection of 100 mg/kg (14 rats), there was no edema in 10 cases; K = 0.96.

In the fourth series of experiments the subcutaneous injection of 50 mg/kg of the chlorinated derivative of mepazine (13 rats) prevented the development of edema in 10 cases; K = 0.85 (average value).

In the fifth series of experiments we administered 50 mg/kg of promethazine (10 rats). In 7 cases edema did not develop, in 1 case it was mild, and in two moderate; K = 1.06 (average value).

In addition to the magnitude of K, the survival of the animals may serve as an index of the effectiveness of the preparations. In the control experiments about half of the animals succumbed in the hour after the administration of the edema-provoking substance. In all cases where we used derivatives of phenothiazine death of the animal was not observed.

Thus all the derivatives of phenothiazine investigated by us possess the ability to delay the development of pulmonary edema. The strongest inhibiting action, from both points of view, of experimental edema is displayed by the intraperitoneal or subcutaneous injection of aminazine in doses of 10 and 20 mg/kg. Subcutaneous injection of 50 and 100 mg/kg of mepazine also inhibits the development of pulmonary edema. The chlorinated derivative of mepazine (100 mg/kg subcutaneously) acts more weakly on adrenalin edema, but no less effectively on edema produced by ammonium chloride.

The experiments with promethazine gave in both cases very contradictory results; in this case statistical reliability is lacking. We therefore limit our discussion to the weaker action of promethazine as compared with the other preparations.

In the literature [1, 2, 3, 4] there are found statements that many narcotic substances can prevent the development of toxic pulmonary edema, but for this a considerable depth of narcosis is necessary. Judging by our data, a state of narcosis is not necessary for the production of the antiedema effect. Derivatives of phenothiazine not possessing narcotic action also appear to be effective in the experimental therapy of acute toxic edema of the lungs.

SUMMARY

Preparations of the phenothiazine group, aminazine, mepazine, a chlorinated derivative of mepazine, and promethazine inhibit the development of experimental toxic edema of the lungs, producted by adrenalin and by NH_4Cl. Aminazine is the most effective preparation, promethazine the least effective.

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* Original Russian pagination. See C. B. translation.

ON THE MECHANISM OF ACTION OF HEXENAL

COMMUNICATION 1. THE ACTION OF HEXENAL ON THE INTEROCEPTORS OF THE INTESTINE

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(Received November 29, 1956. Presented by Academician K. M. Bykov)

In spite of numerous investigations of the pharmacology of hexenal [6, 10, 16], its mechanism of action, both as concerns its basic narcotic effect, and its side effects on the circulation and respiration, remains in many ways obscure.

Certain clinical observations and experimental data are hard to explain on the basis of the generally accepted concept of a direct depressive influence of barbiturates on different portions of the central nervous system (cerebral cortex, reticular formation, spinal cord).

To such facts belong, for example, observations indicating a different effect of intravenous and intraarterial injection of hexenal. V. S. Galkin and N. C. Misyuk [4], carrying out hexenal tests on hypertensive patients, observed that the injection of 10 ml of 10% hexenal into the peripheral portion of the femoral artery did not cause a fall in blood pressure nor a state of narcosis. Intravenous injection of the same dose produced a temporary hypotensive effect and the development of a state of general anesthesia. Repeating these observations on animals, A. G. Bukhtiyarov [3] confirmed them and showed in addition that the narcotic and toxic effect of hexenal was more marked when the narcotic was introduced into the external jugular vein towards the heart than when the same dose was introduced into the carotid artery to the brain. These and many other facts led to the idea of a possible role in the mechanism of hexenal anesthesia of reflex effects from receptors of internal organs.

Direct demonstration of action of various narcotics on the receptors of the carotid sinus was obtained by numerous authors [2, 8, 12, 13, 15]. The electrophysiological investigations of D. Whitteridge and E. Bulbring [17] and later A. S. Paintal [14] demonstrated changes in the condition of the expansion receptors of the lungs following inhalation of ether and chloroform.

In the present work the results of 28 experiments on the action of hexenal on the intestinal interoceptors were observed.

METHODS

We carried out our experiments on cats anesthetized with 20% urethane. We transfused a loop of the small intestine, circulatorily isolated, through the superior mesenteric artery and vein with oxygenated Ringer-Locke solution at 38-39°. The innervation of the intestine was completely intact. We administered hexenal in large doses (50, 100, 200 mg), injecting it into the artery of the loop with a syringe (1-2 ml of a 5-10% solution). We investigated the action of smaller doses under conditions of prolonged perfusion of the organ with the same solution, containing from 2 to 100 mg% of hexenal. We recorded the reflex changes in blood pressure (mercury manometer in the carotid artery) and respiration (Marey's capsule attached to the animal's trachea). In addition, in all experiments we verified the responsiveness of the interoceptors to other chemical preparations (acetyl-

choline, Na Evipan (Bayer, Farbenindustrie) and Avipal (Winthrop Chemical Co.). No important differences in their action on the interoceptors were observed.

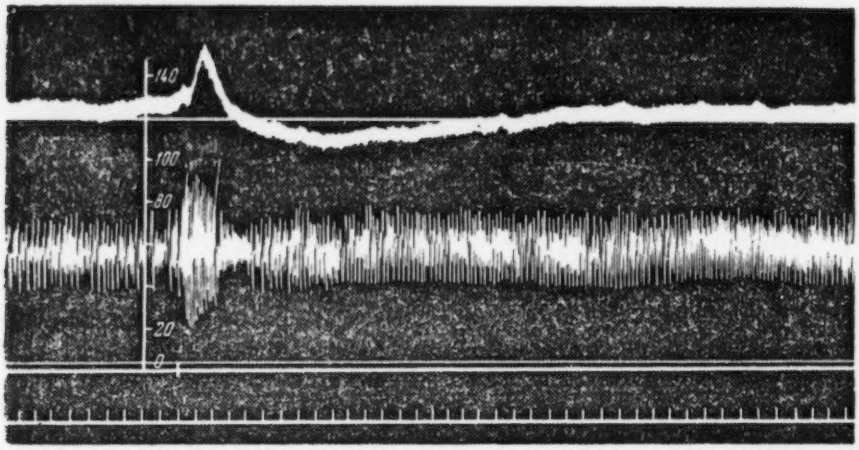

Fig. 1. Reflex changes of blood pressure and respiration in experiments involving injection of Evipan into the blood vessels of an intestinal loop.
Meaning of curves (from top to bottom): Blood pressure in mm of mercury, initial level of blood pressure, respiration, stimulus mark (injection of 1 ml of 10% Evipan), zero line of manometer, time signals (5 seconds).

RESULTS

First we studied (in 7 experiments) the action of 10% hexenal, generally used in surgical and experimental practice. As a rule, the injection into the vessels of an intestinal loop of 1-2 ml of 10% hexenal was accompanied by a two-phase change in blood pressure and respiration (Fig. 1). At the moment of injection of the hexenal the blood pressure rose sharply, then fell below the initial level and returned to normal after various intervals of time (from 1 to 10 minutes). After the initial sharp increase the respiration also decreased somewhat, then was restored to the original level.

Section of the nerves innervating the intestinal loop completely abolished the circulatory and respiratory effect, which demonstrates the reflex nature of these changes.

Later on we attempted to ascertain the minimal active concentration of hexenal, and also study the effect of prolonged perfusion with small doses of the narcotic on the excitability of the interoceptors. Twenty-one experiments were carried out in which the intestine was perfused with hexenal solutions of the following concentrations: 2 mg % (2 experiments), 5 mg % (6 experiments), 10-20 mg % (6 experiments), and 50-100 mg % (7 experiments).

Fifteen to 20 minute perfusion of the intestine with a hexenal solution of concentration 2 mg % had no effect, either on the blood pressure or the amplitude of the respiration. There was only observed a negligible decrease of the vascular pressor reflexes, appearing in response to a small dose of nicotine (2 γ). The reflexes to large doses of the irritant (10 and 20 γ) did not change.

More definite results were obtained on using a 5 mg % solution of hexenal. In spite of the fact that in all experiments reflex changes in blood pressure and respiration were absent following administration of the narcotic, its action was clearly evident in a lowering of the sensitivity of the receptors to other chemical stimuli. One of the experiments is presented in Fig. 2. Three minute perfusion with Evipan in a concentration of 5 mg % [2,3] led to a significant decrease of blood pressure and respiratory reflexes, elicited by the administration of 10 γ of

nicotine [4]. The rate of perfusion in this experiment was 33-34 ml per minute. Subsequently, three minutes after this, 5 mg hexenal in 100 ml of solution was injected into the circulation of the small intestine. Restoration of reflexes was observed only about 28 minutes after washing out the narcotic.

If the duration of perfusion with hexenal of a given concentration was increased to 20-30 minutes, then reflexes to weak stimuli (5-10 γ acetylcholine and nicotine) disappeared completely, reflexes to strong stimuli (50-100 γ) were however preserved, but their intensity was markedly decreased.

More significant alterations of the functional characteristics of the receptor apparatus were observed on increasing the concentration of narcotic in the perfusion fluid to 10-20 mg %. Reflexes to weak stimuli disappeared in the course of 5-10 minutes after the beginning of perfusion, and somewhat later, after about 20-30 minutes, there was observed a complete absence of reflex reactions to the administration of large doses of nicotine and acetylcholine (100 γ). On stopping the perfusion with the "narcotic" solution the reflexes were generally completely restored in the course of half an hour (Fig. 3). As a rule during the action of 10-20 mg%

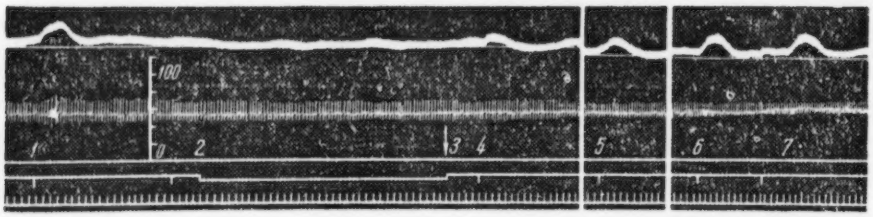

Fig. 2. Reflex changes in blood pressure and respiration inexperiments with perfusion of intestinal loop with Evipan in a concentration of 5 mg %.
Meaning of curves (from top to bottom): blood pressure in mm of mercury, initial blood pressure level, respiration, zero line, records of stimuli (injection of 10 γ of nicotine, 5 γ acetylcholine, time signals (5 seconds).

hexenal on the interoceptors of the intestine the blood pressure dropped somewhat and respiration was slowed.

V. N. Chernigovsky [7] and V. A. Lebedeva [5] observed an analogous effect after injecting a 1-2 % novocaine solution into the intestinal circulation. The electrophysiological investigations of N. Ya. Anikina [1] supported the original explanation, that novocaine, depressing the activity of the interoceptors, produces a decrease in tonic impulses, acting through the afferent fibers of the intestine on the vasomotor and respiratory centers. This same mechanism evidently underlies the reflex lowering of blood pressure and slowing of respiration which are observed during perfusion of the intestine with hexenal.

Further increase of the concentration of hexenal in the perfusion fluid to 50-100 mg % caused complete disappearance of reflexes, both to weak and strong stimuli, after a very short time, from 1 to 5 minutes. Such doses first produced a negligible elevation of the blood pressure (10-20 mm of mercury) and a moderate strengthening of respiration, which then changed to a hypotensive effect and a decrease in amplitude and frequency of respiration.

DISCUSSION

Hexenal, therefore, in very small concentrations (2-5 mg %) acts on the interoceptors of the intestine, producing on prolonged contact a gradual decrease in their excitability. With larger concentrations of hexenal (10 %) there is observed an initial strong irritability of the receptors with a subsequent depression of their activity.

Since there is no basis for suggesting a specific sensitivity of the interoceptors to hexenal, the latter probably exerts from the first moment an equally strong general influence on all the receptors in its path. It is possible that on intravenous injection the receptor fields of the heart and lungs have special significance [9].

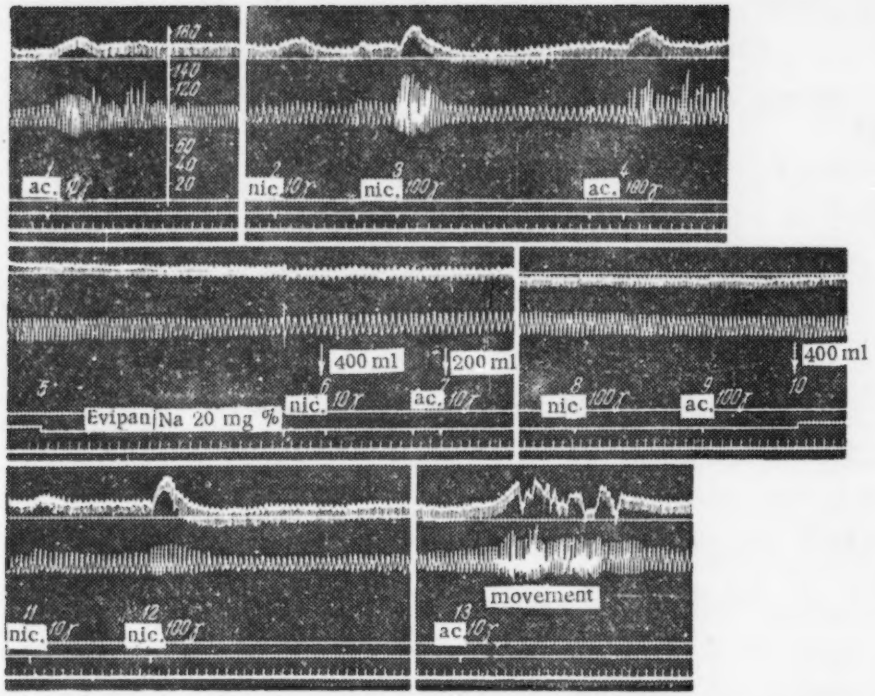

Fig. 3. Reflex changes in blood pressure and respiration in experiments with perfusion of intestinal loop with Evipan in a concentration of 10-20 mg %.
Meaning of curves (from top to bottom): blood pressure in mm of mercury, initial blood pressure level, respiration, records of stimuli [injection of 10 γ acetylcholine (1, 7, 13); 100 γ acetylcholine (4, 9)]; 10 γ nicotine (2, 6, 11); 100 γ (3, 8, 12). Time signals (5 seconds).

being subjected before all others to the action of the narcotic. The prolonged action of hexenal on other interoceptors would depend, naturally, on the rapidity of destruction of hexenal and its excretion.

According to the data of H. Genuit and K. Labenz [11], the narcotic concentration of hexenal in the blood is approximately 1.4-1.5 mg %. We did not succeed in detecting a decrease in the excitability of the receptors of the intestine from the action of solutions of hexenal in concentrations of 2-5 mg %. Taking into account the possible disturbance of the functional state of the reflex apparatus produced under the conditions of the experiment (narcosis, perfusion), it must be recognized that the concentrations of hexenal employed by the authors referred to and by us are quantities of the same order of magnitude.

Consequently the interoceptors present themselves as one of the target organs on which hexenal can act when it is administered to animals or man. Therefore the assumption of a possible role of an interoceptor component in the mechanism of hexenal narcosis receives still further confirmation.

SUMMARY

By perfusing an isolated intestinal loop in cats anesthetized by urethane, reflex changes of blood pressure and respiration produced by hexenal, acetylcholine and nicotine on intestinal interoceptors were studied. Strong (10%) and weak 2-5 mg %) solutions of hexenal affect the interoceptors, lowering their excitability after prolonged contact. A reflex from the interoceptors probably plays a part in the mechanism of hexenal anesthesia.

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** Original Russian pagination. See C. B. translation.

ON THE SELECTIVE EFFECT OF SOME CHEMICAL IRRITANTS ON REFLEXES FROM CHEMORECEPTORS

COMMUNICATION 5. EFFECT OF SUBARACHNOID INJECTION OF STREPTOMYCIN ON REFLEXES FROM CHEMORECEPTORS, MECHANORECEPTORS, AND AN AFFERENT SOMATIC NERVE

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(Received December 28, 1956. Presented by Active Member of the USSR Acad. Med. Sci. Prof. V. N. Chernigovsky)

Streptomycin, when injected intramuscularly or intravenously, exhibits a profound depressing effect on reflexes from the chemocenters of the small intestine, kidneys, spleen, and carotid sinus, as was established by us previously [2, 5, 6]. In contradistinction to this, the antibiotic, under the same experimental conditions, does not exhibit such an effect, or produces it to a much lower degree, if unconditioned reflexes arising from mechanical stimulation of the bladder, stomach, small intestine, or the central portion of the tibial nerves are considered. The above-mentioned property of streptomycin appears in experiments in which blood pressure and respiration serve as the indicator, as well as in experiments in which the leukocyte picture of the peripheral blood serves as the indicator. These observations led us to the conclusion that streptomycin exhibits a selective effect on the reflexes on the chemoreceptors by way of a direct influence of the central portions of the afferent part of the reflex arc. Taking account of the fact that the antibiotic, injected intramuscularly or even intravenously, penetrates to the brain in insignificant amounts [1, 4, 7, 8], it was necessary to assume a high sensitivity of the corresponding parts of the central nervous system to streptomycin directly. We believe that this assumption can be checked to a certain extent by experiments in which streptomycin is introduced into the subarachnoid space of the brain. If following such a mode of administration the antibiotic exhibited its characteristic influence on the interoceptor reflexes, rapidly and in moderate doses, then the assumption that it acts on the central part of the reflex arc would receive additional confirmation.

METHODS

We carried out the experiments in cats anesthetized with urethane. We recorded the respiration and the blood pressure in the carotid artery. We made a record of the reflexes on smoked kymograph paper.

The chemoreceptors of the carotid sinus were stimulated by the intravenous injection of 0.15-0.2 ml of 0.15% cytotone.* We produced the reflexes of the chemoreceptors of the intestine by the method of V. N. Chernigovsky. We produced the reflexes of the chemoreceptors and mechanoreceptors of the pericardium by the method of C. K. Drinker [8].

We injected streptomycin (the calcium chloride complex) into the subarachnoid space. By the method described by V. S. Galkin [3], with the aid of a special needle, we made a puncture in the atlanto-occipital membrane. Then with a syringe we removed fluid and injected the corresponding volume (1-2.5 ml) containing various doses of streptomycin.

* Transliterated. Probably a Russian trade name.

RESULTS

In the first series of experiments the influence of subarachnoid injection of streptomycin on the reflexes from the chemoreceptors of the carotid sinus was studied.

First of all it was established that the reaction of the animals to subarachnoid injection of streptomycin depends both on the concentration of the preparation injected and on the dose. Following injection of the antibiotic in a concentration of 40,000 units per ml and higher, there generally ensued cessation of breathing and a considerable lowering of blood pressure. In a series of experiments the animals succumbed. This impelled us to employ streptomycin in a concentration of 25,000 units in 1 ml physiological saline. Doses of streptomycin up to 50,000 units with moderate concentrations of the antibiotic in the injected fluid as a rule were well tolerated by the animals. There was observed merely a slowing of respiration and diminution of the respiratory motions. Doses exceeding 50,000 units led in some cases to an alteration of the respiratory rhythm and in some to cessation. The blood pressure also declined. However it should be noted that in separate experiments animals tolerated doses of streptomycin greater than 55,000 units.

In control experiments on the influence of the injection of saline alone into the subarachnoid space the reflex to cytotone did not change, rarely became somewhat, though negligibly, weaker.

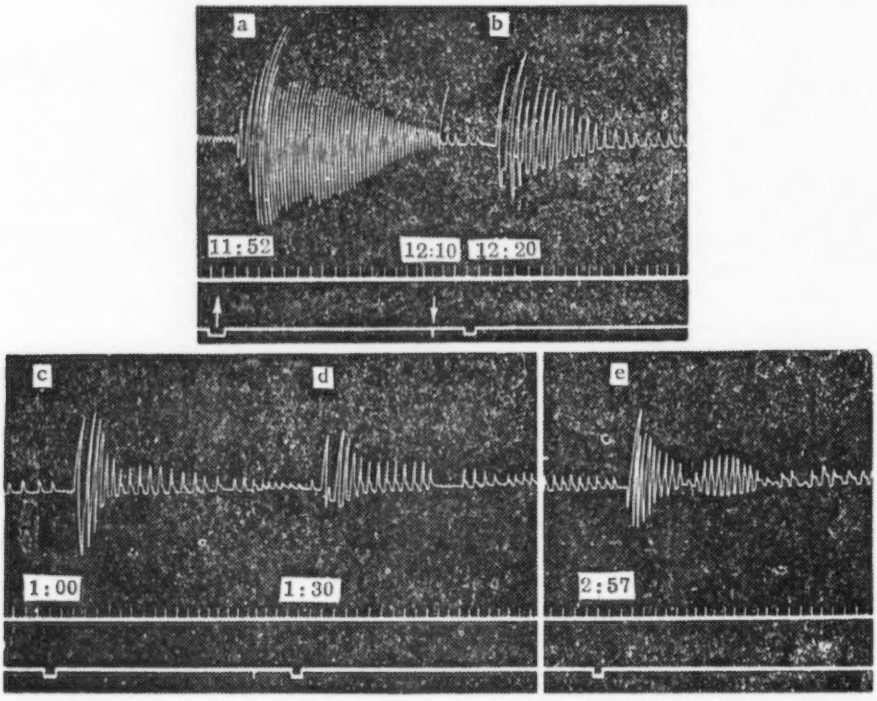

Fig. 1. Effect of subarachnoid injection of streptomycin on the respiratory reflex produced by cytotone. a) reflex to cytotone before the injection of streptomycin, b, c, d, e) reflex to cytotone after injection of 52 thousand units of streptomycin. Experiment No. 275, January 22, 1955. Cat weighing 2.8 kg. Significance of curves (from top to bottom); respiration, time signals (5 seconds), records of stimuli: ↑ - reflex before injection; ↓ - reflex after injection.

Under the influence of subarachnoid injection of streptomycin (30-40-50-60,000 units) the initial reflex from the chemoreceptors of the carotid sinus produced by cytotone was sharply depressed in 9 out of 10 experiments. Only in one experiment was this effect negligible.

Even at the first check of the reflex to cytotone, which in the majority of experiments was carried out 7-10 minutes after the subarachnoid injection of streptomycin, there was evident a distinct depression of the respiratory reaction. This was expressed as a considerable diminution of the increase in amplitude and frequency of the respiratory motions and also a considerable shortening of the time of the respiratory reaction (Fig. 1).

In separate experiments the reflex to cytotone was tested 3-4 minutes after the injection of streptomycin. Even in these cases there was a marked depression of the respiratory reflex.

About 20-25 minutes after the subarachnoid injection of the antibiotic the depression of the respiratory reaction to cytotone in most of the cases reached its maximum. In experiments the maximum depressive effect of the streptomycin appeared a little later, 30-40 minutes after the injection of the preparation.

A distinct depression of the reflex to cytotone generally lasted the whole period of observation, i.e., 1 $^1/_2$-3 hours.

In 13 experiments of a second series we investigated the influence of streptomycin on the reflexes arising from stimulation of the chemoreceptors of a perfused intestinal loop. The calcium chloride complex of streptomycin was injected into the subarachnoid space in a dose of 17.5-36,000 units per animal in 1-1 $^1/_2$ ml of saline. Before and after injection of the antibiotic we established the magnitude of the reflexes from the chemoreceptors of the small intestine to CO_2, acetylcholine, and nicotine.

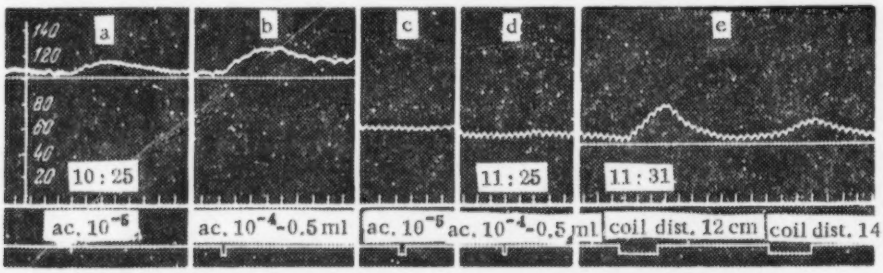

Fig. 2. Effect of subarachnoid injection of streptomycin on reflexes of chemoreceptors of small intestine. a, b) original reflex; c, d) under the influence of streptomycin (injected into the subarachnoid space at 10:33 in dose of 30 thousand units) the reflexes disappeared; e) under these conditions the excitability of the tibial nerve remains high (threshold reflex produced in control experiments with an induction coil distance of 14-17 cm) Experiment No. 337 on March 1, 1955. Cat weighing 3.4 kg. Meaning of curves (from top to bottom): Blood pressure, time signals (5 seconds), records of stimuli.

Under the influence of the injection of streptomycin into the subarachnoid space of the brain the reflexes from the chemoreceptors in the greatest portion of cases disappear completely or are decreased more than 60% (Fig. 2).

In 4 experiments there ensued complete suppression of all reflexes arising from stimulation of the chemoreceptors of perfused intestine. A marked effect developed 3-5-13 minutes after injection of the antibiotic and remained steady for 1 $^1/_2$-2 hours of observation. It is important to note that the depression of the reflexes from the chemoreceptors was not caused by a paralyzing effect of the antibiotic on the vasomotor center. This latter is confirmed by experiments in which it was established that in the presence of complete depression of the reflexes from the chemoreceptors of the small intestine the excitability of the vasomotor center to impulses arising from the stimulation by electric current of the tibial nerve remained sufficiently high (Fig. 2). In the remaining cases streptomycin produced a not completely equal depression of the reflexes from the chemoreceptors when given various stimuli.

In 8 experiments the effect of the antibiotic on reflexes produced by acetylcholine in various concentrations was observed. In 4 experiments the effect of streptomycin on reflexes produced by acetylcholine in dilutions of 10^{-5} and 10^{-6} was studied. Out of these the reflexes disappeared in three, and in one was decreased by 50%. In 4 cases acetylcholine was employed in a dilution of 10^{-4}; in two of these the reflex was completely suppressed, in one case decreased by 75% in one 22%. Acetylcholine in a dilution of 10^{-3} was employed in 6 experiments. In one of these the reflex disappeared, in three it decreased by 60-66%, in two by 39 and 20%.

In 5 experiments the reflexes from the chemoreceptors were produced by nicotine (10^{-6} and 10^{-5}). Under the influence of streptomycin in one case the reflex disappeared completely, in the others it decreased by 54-66-72-88%.

The reflex to CO_2, tested in 2 experiments, also decreased: in one by 84%, in the other by 66%.

Thus, in spite of the unequal stability of different reflexes in different experiments, in all observations without exception subarachnoid injection of streptomycin produced pronounced depression of the reflexes from

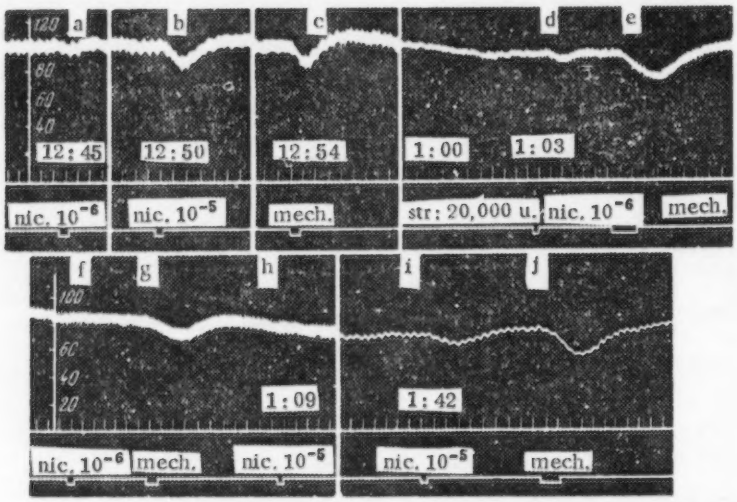

Fig. 3. Effect of subarachnoid injection of streptomycin on reflexes from chemoreceptors and mechanocenters of the pericardium. a,b,c) original reflexes; d,e,f,g) profound depression and subsequent partial recovery of reflexes from chemoreceptors after subarachnoid injection of streptomycin (20 thousand units at 1:00); h,i,j) at this time the reflex from the mechanocenters almost undiminished in magnitude. Experiment No. 409 on July 13, 1955. Cat weighting 3.8 kg. Meaning of curves as in Fig. 2.

the chemoreceptors. This effect, as in the first group of observations, developed quickly, in the course of the first 5-10-15 minutes, barely 20-30 minutes after injection of the streptomycin into the subarachnoid space. On prolonged observation of the reflexes referred to, a marked stability of the depressive effect of the antibiotic on the reflexes from the chemoreceptors was noted when the preparation was injected into the subarachnoid space.

In the third and last series of experiments we studied the effect of subarachnoid injection of streptomycin on the reflexes from the chemo- and mechanocenters of the pericardium. We injected streptomycin in the same dose as in the previous experiments. It was established that subarachnoid injection of streptomycin produces profound depression of the reflexes from the chemoreceptors of the pericardium, which sets in soon after the injection of the antibiotic. In contrast to this the reflexes from the mechanocenters of the pericardium, in

our observations, did not change or decreased negligibly, which is clearly visible on the kymogram, presented in Fig. 3.

Thus in all three series of experiments it was demonstrated that on injection of streptomycin into the subarachnoid space of the brain, blocking of the reflexes from the chemocenters of various reflexogenic zones ensues. It results from the employment of moderate doses of the antibiotic and maintains itself very constant. The depressive action of streptomycin on the reflexes from the chemoreceptors was not accompanied by significant alteration in the reactivity of the vasomotor center to the reflex influence from the mechanoreceptors and a peripheral afferent somatic nerve.

These conclusions, following from our observations, allow one to conclude that streptomycin, when introduced into the central nervous system, exhibits a direct blocking action on the central portion of the afferent part of the reflex arc, beginning with the tissue chemoreceptors. Therefore the above mentioned assumption as to the point of action of streptomycin on the interoceptor reflex arc receives additional confirmation.

SUMMARY

Data are reported on the effect of subarachnoid injection of streptomycin on reflexes from the chemoreceptors of the carotid sinus, small intestine, and pericardium. Anesthetized cats were used in the experiments. Reflexes from the zones mentioned to chemical stimuli were profoundly depressed by streptomycin. Reflexes from mechanoreceptors, on the contrary, were almost unchanged. The excitability of the vasomotor center, when the tibial nerve was stimulated, remained quite high. It is concluded that streptomycin blocks directly the central portions of the afferent part of the chemoreceptor reflex arc.

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* In Russian.

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CHANGES IN THE BIOELECTRIC POTENTIALS OF THE CEREBRAL CORTEX CAUSED BY DETELEN*

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(Received November 3, 1956. Presented by Academician A. D. Speransky)

Detelen (the dicholine ester of succinic acid), given to us by Prof. A. L. Midzhoyany, in whose laboratory it was first synthesized in 1947-1948, is regarded as diacetylcholine and posseses powerful curareform properties [4].

Its pharmacodynamics were demonstrated in investigations carried out in the laboratory of M. Ya. Mikhelson. R. S. Rybolovlev [4] investigated the effects of diacetylcholine on striated muscle and blood pressure. S. N. Vishnyakov, M. Ya. Mikhelson, E. K. Rozhkova and R. S. Rybolovlev [2] carried out a comparative study of diacetylcholine in regard to its curareform properties, its effect on blood pressure, etc. Finally, S. M. Vishnyakov [1] showed that diacetylcholine exhibits a stimulating action on the N-choline receptors of the blood vessels of the reflex zone.

The effect of detelen on the state of the central nervous system, in particular on its higher centers, remains unelucidated.

Studying the effect of detelen on spinal reflexes, R. S. Rybolovlev came to the conclusion that "detelen in curarizing doses exhibits no effect on the central nervous system."

We studied the effect of detelen on the bioelectric activity of various regions of the cerebral cortex of dogs. The purpose of our investigations was to estimate the functioning of the cerebral cortex during the development of the curareform effect by changes in its electrical activity.

METHODS

The electrical activity of the cortex was recorded, using a 4-stage amplifier and a recording oscillograph. We led off the biopotentials from the occipital, parietal, temporal and frontal regions of the cortex.

In addition to the bioelectric currents, we recorded also the blood pressure, respiration, and body temperature.

Since the animals became absolutely motionless only when breathing ceased, we gave the animals artificial respiration, which was carried out with the help of the appropriate apparatus, which was present in all the experimental rooms and which was attached to the trachea by a rubber tube.

Two series of experiments were carried out. In the first series, consisting of 10 experiments, the effect of detelen in a dose of 0.8-1 mg/kg was investigated.

In agreement with the findings of R. S. Rybolovlev, a sharply expressed curarizing effect was observed following the administration of doses of 5 to 10 mg/kg. Therefore in the second series (12 experiments) we investigated the bioelectric activity of the cerebral cortex after the injection of large doses (5-10 mg/kg) of

* Russian trade name.

detelen. In all cases a 1% solution of detelen was injected intramuscularly.

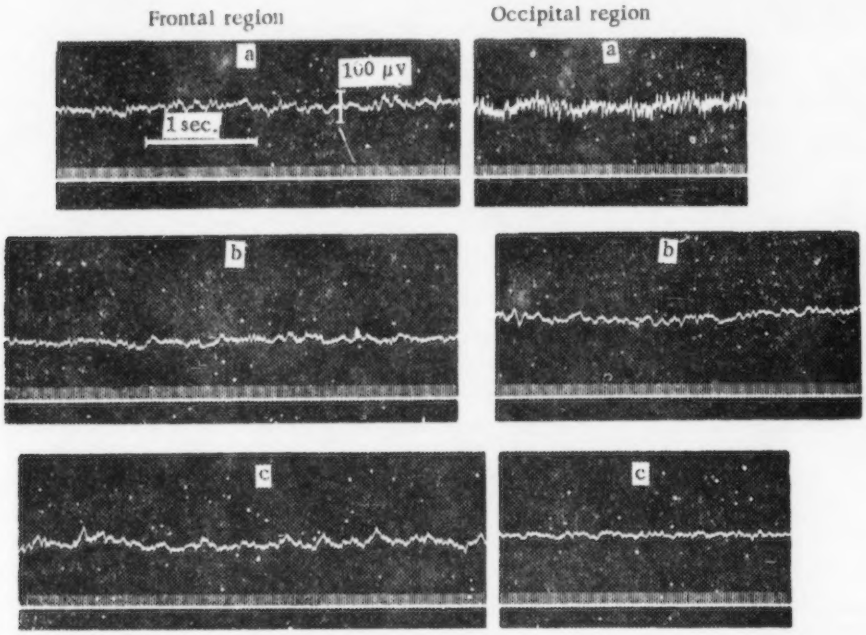

Fig. 1. Changes in the bioelectric activity of the cerebral cortex after injection of detelen. a) before injection of detelen; b) about 15 minutes after injection of 0.8 mg/kg of detelen; c) about 40 minutes after injection of detelen (dog still completely motionless).

Changes in the electroencephalogram (EEG) appeared within 2-3 minutes after intramuscular injection of detelen in large doses, and about 10-15 minutes after the injection of small doses of the preparation.

Analysis of the EEG provides evidence that in the animals curarized with detelen a depression of the dominant rhythm of biopotentials is observed in all regions of the cerebral cortex which were tested, and as would be expected the degree of depression of the dominant rhythm varies according to the dose of detelen, i.e., the extent and duration of the curarizing effect.

As can be seen from Fig. 1 (Experiment No. 4), detelen brings about a decrease in amplitude of the oscillations of cerebral cortex potential, and as would be expected the depression is more distinct in the occipital region of the cortex. About 40 minutes after administration of detelen (c), the bioelectrical activity of all regions recovered completely, but in the occipital region rapid oscillations of low amplitude were observed. If before the administration of detelen the amplitude of the dominant rhythm of the occipital region was 40-50 μv, then about 50 minutes after injection it was 10-15 μv.

Depression of the biopotentials sets in about 10-15 minutes after administration of the detelen; before this there are no important changes in the EEG.

The second series of experiments showed that detelen in doses of 5-10 mg/kg produces stronger and earlier alteration of the bioelectric activity of the brain. As a rule in about 3-5 minutes there is observed a sharp alteration in rhythm, and in about 10 minutes depression of the bioelectric activity of all areas sets in. In Fig. 2 (Experiment No. 3) there are presented electroencephalograms in various stages of immobilization of the dogs.

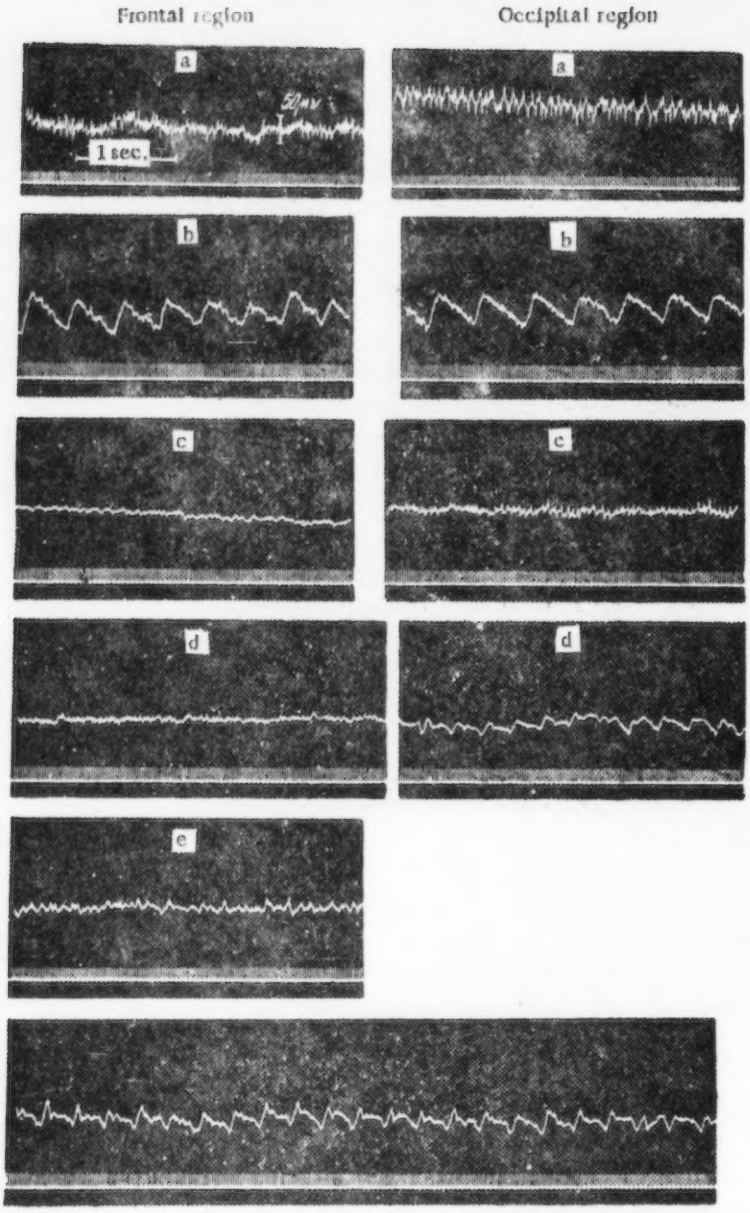

Fig. 2. Change in bioelectric potentials of the cerebral cortex after administration of 5 mg/kg of detelen. a) before injection; b) about 3 minutes after injection; c) about 1 hour after injection; d) about 1 ½ hours after injection; e) about 2 hours after injection.

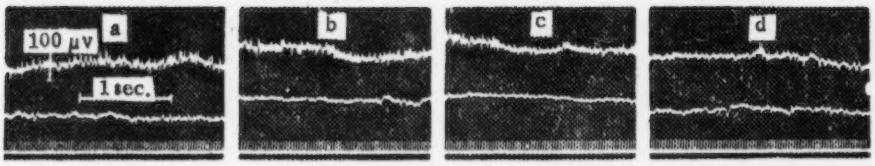

Fig. 3. Effect of detelen on the bioelectrical activity of the parietal region of the cerebral cortex. a) before injection; b) about 30 minutes after injection; c) about 2 hours after injection.

Comparison of the EEG recorded about 3 minutes after administration of the detelen (b), with the original (a) could lead to the conclusion that in that time detelen caused the appearance of slow regular waves, with the superposition of high frequency oscillations of low amplitude. These changes were recorded from all regions of the cortex, including the occipital. About an hour (c) and about 1 $^{1}/_{2}$ hours (d) after administration of the detelen there was observed a sharp depression of the dominant rhythm. Two hours after the administration of detelen the bioelectric activity of all regions tended to be restored (e), but in the EEG of the occipital region slow waves predominated.

As shown above, detelen causes a depression of the bioelectric activity of all regions of the cerebral cortex.

It is evident from the encephalogram of Experiment No. 8 (Fig. 3) that detelen produces a sharp depression of the biopotentials of the parietal region. It should be noted that the depression is always prolonged, as the EEG record about 2 hours after administration of detelen (c) shows the same changes as after 30 minutes (b).

Aside from this, we have established that as the curarizing effects of detelen wear off (muscle twitches appear in the dogs), the bioelectrical activity of the cortical regions studied is almost completely restored.

Blodd pressure records showed that detelen, as a rule, did not produce substantial alterations in the hemodynamics.

The body temperature of the immobilized animals fell.

In the first series of experiments the body temperature of the dogs went down by 0.5-1°, and in the second series the temperature went down by 1-15°.

SUMMARY

The effect of detelen on the bioelectric activity of various areas of the cerebral cortex was studied. The effect of detelen depends on the dose. Small doses resulting in a marked curareform effect affect the electric activity of the brain only slightly, while large doses cause significant inhibition of the activity of the cerebral hemispheres.

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ONCOLOGY

NORMAL AND PATHOLOGIC ANTIGENS IN HUMAN CARCINOMA

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There is now no doubt that the cells of malignant tumors originate from normal cells and tissues. This is proven by the well-known fact that tumors may be obtained by treating normal tissues with different carcinogenic substances and also by the fact that normal tissue can be transformed into tumorous tissue in a tissue culture [10].

The fact that the biochemical and antigenic properties of cancerous tissue are extremely similar to those of normal tissue in man is another proof of the above. The tissues of cancerous tumors have been found to possess the same antigenic substances as normal, nontumorous tissues. It has been established that the same specific [4], blood-group – A, B, AB [5, 11, 13], type – M, N, MN and Rh antigens [6] are found in both the tissues of cancerous tumors and the cells and tissues of normal organs.

It has also been established that each cancerous tumor contains the same set of antigens (specific, blood-group, type) as do the normal cells and tissues in the individual with that tumor. These normal antigens, i.e., the antigens present in normal cells and tissues, appear at an early stage of human embryonic development, remain qualitatively the same throughout the individual's life and are passed on to his descendants, i.e., are the normal, isogenetic, antigenic features of the individual.

Cancerous cells originating from normal cells and tissues retain the ability of the former to synthesize blood-group, typo- and species-specific antigenic substances. Therefore, tumors vary as to their specific, isogenetic, antigenic properties in different people, as do normal tissues. However, research has also shown [1, 2, 7, 9] that cancerous cells can synthesize new antigenic substances as well, specific for the cancerous tumor only – pathological, carcinomatous antigens.

There are some researchers, however, who do not agree that the origin and development of a tumor is attended by the appearance of new antigens in it, which did not exist in the body before the tumor. These authors [12] believe that the differences between the antigenic properties in tumorous and in normal tissues are due to the fact that the body with the tumor is genetically different from other individuals providing the normal organs to be used as the control.

There is some basis for this opinion, since the methods usually used do not make it possible to exclude the existence of genetic, antigenic differences between tumors and normal tissues. Actually, tumor antigens have been compared with antigens obtained from the normal tissues of other individuals in immunological experiments with both transplanted tumors and spontaneous or carcinogenic-induced tumors. Under these conditions, normal isogenetic differences could be taken for specific antigenic differences.

To prove this hypothesis, we decided to make a comparative study of the antigenic properties of cancerous tumors and normal tissues from the same, isogenetically identical material.

Z. I. Rovnova [8] made a study similar to ours on experimental animals with implanted tumors.

EXPERIMENTAL METHODS

The object of our study was to compare the antigenic properties of cancerous tumors with those of undiseased portions of organs taken, as mentioned above, from the same individual. Tumors and healthy tissues from 18 cadavers which had died from cancer were examined repeatedly.

The antigens from the tumors and healthy tissues were prepared in a salt solution by an earlier described method [7]. Immune, antitumor sera were obtained from three kinds of animals – rabbits, goats and horses.

The horses were immunized with a mixture of salt extracts from metastasized-to-the-liver cancer of the stomach, cervix and breast. The goats were immunized with salt extracts from metastasized-to-the-liver cancer of the stomach and gall bladder. The antigens used to immunize the horses and goats were first processed with glycerine to inactivate bacterial flora. The rabbits were immunized with salt extracts from metastases to the liver of cancer of the stomach.

Before the experiment, the rabbit and goat sera were subjected to absorption according to a method we developed [7] in order to remove incidental, nonspecific antibodies. The horse serum (in a definite dilution) reacted specifically with the antigen from the tumors, but did not react with the antigens from the normal tissues. When the serum reacted nonspecifically with extracts from the normal organs, we had to treat it by absorption before the experiment in the same way. We examined the antigenic properties of tumors and normal tissues by means of a complement fixation reaction at a temperature of 37°.

EXPERIMENTAL RESULTS

Table 1 shows the results obtained from the comparative study of the antigenic properties of tumors and of normal tissues taken from 6 individuals. It is clear from the table that the antitumor immune sera reacted with the salt extracts from cancerous tumor tissues, but did not react with the extracts from normal organs obtained from the same people. These experiments indicated that the tissues of cancerous tumors contain special antigenic substances, present only in the tumors and not in the tissues of the normal organs. We could not find the "cancer" antigen in the tissues of the spleen, liver, kidneys, lungs, myocardium or brain which had been obtained from cancer patients.

Therefore, from these experiments, it follows that the ability of tumors to react with the corresponding antitumor sera does not depend on the antigens which the tumors have in common with the normal organs, but is determined by the "cancer" antigens, which are only found in the tumors.

As Table 1 shows, the tumors' ability to react with the corresponding sera is not associated with the localization of the tumors or with the blood-group antigens contained in them. The antitumor sera reacted with antigens from various tumors of primary cancer of the liver and lungs and also with metastases of variously placed cancerous tumors to the liver and lungs. These sera, however, did not react with water-salt extracts from 12 other forms of tumors (Table 2).

These experiments, therefore, confirm the fact mentioned earlier [3] that cancerous tumors from different people can differ qualitatively as to specific antigenic properties. In our experiments (see Table 2), the antitumor sera reacted with the antigens of some tumors, but did not react with the antigens of others. What produces these differences in specificity found in the cancer antigens has not yet been determined, however. As Table 2 shows, one cannot connect these differences with the localization of the tumor. One must note, though, that metastases of the same tumor to different organs – the liver and lungs (see No. 8 and 12) – reacted similarly in our experiments.

As well as studying the tumors and normal tissues with antitumor sera, we also studied the antigenic properties of these tissues with sera to normal human organs, the liver and spleen.

Table 3 shows that the organo-specific sera which we obtained to the spleen and liver reacted only with salt extracts from the corresponding normal organs, i.e., with livers or spleens taken from people who had died of cancer, and did nor react at all with salt extracts from cancerous tumor tissues. None of the organo-specific antigens present in the normal organs, the liver and spleen, were found either in the extracts from the primary cancer of the liver tissue or in the extracts from metastasized-to-the-liver cancer of the stomach tissue, in spite of the fact that these extracts contained the pathologic "cancer" antigens (see Table 1, No. 693 and 694).

TABLE 1

Antigenic Differences Between Normal Tissues and Cancerous Tumor Tissues in Man

Case number	Name bl. gr. cancer victims	Tumor description	Dilution antitumor sera	Experimental tissues: Tumor	Spleen	Liver	Lung	Kidneys	Heart	Brain
633	M-v AB	Metastasis-to-liver cancer of the pancreas	1 : 20	++++	—	—				
			1 : 40	+++(+)	—	—				
			1 : 80	+	—	—				
641	S-va 0	Metastasis-to-liver cancer of the pancreas	1 : 20	+++++	+	+				
			1 : 40	++	—	—				
			1 : 80	+	—	—				
649	K-v 0	Lung cancer	1 : 20	+++++	—		—		±	
			1 : 40	+++	—		—			
			1 : 80	++	—		—			
693	Zh-va 0	Metastasis-to-liver cancer of the stomach	1 : 80	+++++			—	—	—	
			1 : 160	++	—		—	—	—	
			1 : 320	+	—		—	—	—	
694	Yu-ya A	Primary cancer of the liver	1 : 40	+++++	+	—	—	±		
			1 : 80	+++(+)	—	—	—	—		
			1 : 160	++	—	—	—	—		
855	F-va	Metastasis-to-liver cancer of the esophagus	1 : 80	+++++	—	—	+	—		—
			1 : 160	+++++	—	—	—	—		—
			1 : 320	+++	—	—	—	—		—
857	0	Metastasis-to-lung cancer of the esophagus	1 : 80	+++++	—	—	±	—		—
			1 : 160	++	—	—	—	—		—
			1 : 320	+	—	—	—	—		—

Meaning of symbols used: + different degrees of positive reaction; ± doubtful reaction.

Note: Cases No. 633, 641, 649, 693 and 694 designate experiments done with antitumor serum obtained from the horse; No. 855, from the rabbit; No. 857, from the goat.

These experiments, therefore, indicate that the pathologic "cancer" antigens are immunologically isolated from normal, organo-specific antigens. When primary cancer or metastases developed in the liver, the normal, healthy liver tissues surrounding the tumor did not contain the specific "cancer" antigen in a quantity practicable for immunological analysis. The "cancer" antigen only appeared in the cells affected with cancer.

Therefore, our studies showed that in spite of the isogenetic antigens shared by normal and cancerous tumor tissues, the cancerous tissues characteristically also possess special "cancer" antigens, specific to these pathologically changed tissues. These antigens are immunologically isolated from normal (blood-group, type and organo-specific) antigens and only appear in the body during the origin and development of a cancerous tumor. There are no organo-specific antigens of the liver present in the tissue of a primary cancer of the liver or in metastases to the liver. Some forms of tumors have immunologically similar "cancer" antigens, but other forms are characterized by "cancer" antigens completely different from each other. The similarity or difference of the "cancer" antigens does not depend on the localization of the tumor nor on the specificity of the normal antigens contained in it.

TABLE 2

Discovery of the Specific Cancer Antigen in Variously Located Tumors

Tumor number	Description of tumor	Number of cases	From them with the antitumor sera	
			Reacted	Did not react
	Lung cancer	3	1	2
	Primary cancer of liver	1	1	
3	Cancer of stomach	1	—	1
4	Cancer of prostate	1	—	1
5	Bronchial cancer	1		1
6	Metastasis-to-liver cancer of pancreas	4	2	2
2	Metastasis-to-liver cancer of stomach	2	1	1
8	Metastasis-to-liver cancer of esophagus	2	1[1]	1
9	Metastasis-to-lung cancer of stomach	1	—	1
10	Metastasis-to-liver cancer of large intestine	1		1
11	Metastasis-to-lung cancer of breast	1	—	1
12	Metastasis-to-lung cancer of esophagus	1	1[1]	—

[1] Metastases of tumors from the same patient

TABLE 3

Tissues from Normal Organs and from Tumors Examined for the Presence of Organo-specific Antigens

Case No.	Name bl. gr. cancer victims	Localization of tumor	Organo-specific sera in relation to:	Dilution of serum	Results of examining antigens from tissues		
					Tumor	Spleen	Liver
691	Zh-va 0	Metastasis-to-liver cancer of stomach	Spleen	1 : 10	—	+ + + +	
				1 : 20	—	+ + +	
				1 : 40	—	+	
696	Yu-ya A	Primary cancer of liver	Liver	1 : 50	—		+ + + +
				1 : 100	—		+ + + +
				1 : 200	—		+ +
				1 : 400	—		+
698	Yu-ya A	Primary cancer of liver	Spleen	1 : 40	—	+ + + +	
				1 : 80	—	+ + + ( )	
				1 : 160	—	+ +	
				1 : 320	—	+	

SUMMARY

Comparative studies of antigenic properties of cancerous tissues and of normal organs in one and the same subject demonstrated special "cancer" antigens in cancerous tissues, specific for these pathologically altered tissues.

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THE EFFECT OF DOPAN* ON VARIOUS ANIMAL TUMORS

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The works of L. F. Larionov and G. N. Platonova [4] have shown that the new, antitumorigenic preparation, 4-methyl-5-di (2-chlorethyl)-aminouracil (dopan) causes the steady resolution of fusiform sarcoma-45 in 90% of the rats treated. The great influence of dopan on this strain of tumor suggested that its effect on other animal tumors be studied. For this purpose, as well as sarcoma-45 [2], we used ten different strains of transplanted tumors, namely: rat tumors – M-1 and Tarashchansky's polymorphocellular sarcomas [9, 7] and cancer of the uterus (Geren's tumor); mouse tumors – Crocker's sarcoma, Ehrlich's tumor (ascitic variant transplanted subcutaneously) [8], adenocarcinoma of the mammary gland (RSM strain) [6], solid carcinoma of the mammary gland (MAP strain) [1], transplanted cancer of the liver (hepatoma XXII) [3], mouse leukosis LIO-1 [5] and rabbit, Brown-Pearce carcinoma [8]. The antitumorigenic effect of dopan was also examined on rat sarcomas induced by 9, 10-dimethyl-1, 2-benzanthracene. The experiments were done on 274 rats, 506 mice and 31 rabbits.

EXPERIMENTAL METHODS

We did not begin the dopan therapy immediately after transplantation of the tumors, but waited until the tumors had reached a definite size. The rat tumors weighed about 1 g and the mouse tumors 0.1 g, i.e., each tumor was about 1% of the animal's body weight when the treatment was begun. In the experiments with the LIO-1 strain of leukosis and with the Brown-Pearce tumor, we began to administer the preparation a few hours or a day after transplantation. Dopan was administered to the rats per os in a dose of 0.3 mg per 1 kg of body weight daily, to the mice, in a dose of 0.4 mg per 1 kg of body weight daily and to the rabbits, in a dose of 0.75 mg per 1 kg of body weight every 72 hours. The treatment lasted 15-20 days, depending on the individual growth of the various tumors.

The experimental results were estimated according to the percentage of growth inhibition, calculated from the average weight of the tumors in the control and experimental groups. The data obtained was proven statistically by the Fisher-Student method. The percent of inhibition was considered true if the index, p, calculated according to this method, equaled 0.950 or more.

The criterion of the preparation activity in the experiments with leukosis was the average duration of the mice's life. In the experiments with the intravenous implantation of the Brown-Pearce tumor, the degree to which the internal organs were affected by the tumorous nodes was determined.

EXPERIMENTAL RESULTS

Table 1 gives the average data obtained from several series of experiments. Table 1 shows that sarcoma

* Russian trade name.

45 and Tarashchansky's sarcoma, in that order, were the most sensitive to dopan. The growth of Tarashchansky's sarcoma was inhibited 93% by dopan, and, in some of the rats (about 18%), dopan caused the resolution of the tumor.

Dopan inhibited the growth of the other experimental tumors, but did not cause their resolution. Table 1 gives the tumor strains in the order of their growth inhibition.

The study of dopan's effect on mouse leukosis, which was done on 150 mice, showed that this preparation had a weak antitumorigenic effect and prolonged the life of the mice in the experimental group by 20% as compared with the control.

When the Brown-Pearce tumor was implanted intravenously in the control rabbits, numerous tumors developed in the lungs, liver, kidneys and other internal organs, which led to the death of the animals on the 20th day after implantation. In two experiments on 31 rabbits, it was established that dopan considerably inhibited the development of the Brown-Pearce tumor in the internal organs (Table 2).

Table 2 shows that there were more tumor nodes in the internal organs of the control rabbits than in those of the experimental animals.

Therefore, the studies conducted indicate that dopan has a wide range of antitumorigenic action, affecting 12 different animal tumors. However, its degree of effectiveness varies. The antitumorigenic action of dopan was strongest on the two rat sarcomas – the fusiform sarcoma-45 and the polymorphocellular Tarashchansky's tumor – and on one rat carcinoma (Geren's tumor). Dopan moderately inhibited the development of the

TABLE 1

Effect of Dopan on Various Animal Tumors

Name of strain	Number of animals	Percent of inhibition	Proof of difference (criterion: p)
Sarcoma-45	80	100	1,0
Tarashchansky's sarcoma	40	93	1,0
Carcinoma of the uterus (Geren's tumor)	70	87	0, 978
Carcinoma of the mammary gland (MAP)	80	73	1,0
Ehrlich's tumor	117	69	0,99
M-1 sarcoma	70	64	1,0
Adenocarcinoma of the mammary gland (RSM)	80	57	1,0
Crocker's sarcoma	45	53	1,0
Induced rat sarcomas	14	51	0,877
Transplanted cancer of the liver (hepatoma XXII)	35	37	0,954

Brown-Pearce tumor and also moderately affected the growth of the polymorphocellular sarcoma M-1, the diffuse Ehrlich's tumor, Crocker's sarcoma, MAP carcinoma of the mammary gland and RSM adenocarcinoma of the mammary gland. A weak therapeutic effect was observed on cancer of the liver, mouse leukosis and on the induced rat sarcomas. Our experimental data does not permit the conclusion that the different degrees of sensitivity to dopan of the various tumors is explained by the difference in the histological structure of the tumors, since both epithelial and connective tissue tumors were found among the highly, moderately, and weakly sensitive strains. One must not exclude the possibility that still unstudied biochemical properties of the different tumor strains may have some bearing on the difference in the sensitivity of the various tumors to dopan.

TABLE 2

Effect of Dopan on Brown-Pearce Tumor

Control					Experiment				
No. of rabbits	Lungs	Liver	Kidneys	Other organs	No. of rabbits	Lungs	Liver	Kidneys	Other organs
Experiment I									
185	++++	++++	+	0	188	+	+	+	0
135	+++	++	0	0	77	++	+	0	0
186	++	++	0	0	73	0	0	0	0
137	++	++	0	0	78	0	0	0	0
198	++++	0	0	0	136	0	0	0	0
					147	0	0	0	0
Experiment 2.									
31	++++	++	+++	+	61	+++++	+++	++	0
80	+++	+	+++	++	53	+++	+++	+++	0
41	++++	+++	+++	+	77	+++	+++	++	0
16	++++	++++	++	+	71	+	++	+++++	++++
83	++++	+++	+++	0	92	+	+	++	0
44	++++	++++	++++	0	78	+++	0	0	+
70	+++	+++	++++	+++	15	+	0	+	+
54	++	0	++	+	131	+	+	0	+
79	+++	0	0	0	95	+	+	0	0
20	+	0	0	0	22	0	0	0	+

Note: Intensity of tumorous affection in internal organs is designated as follows: + single tumor nodes (2-3); ++ a few tumor nodes (up to 10); +++ tumor nodes occupying 2/3 of the organ; ++++ tumor nodes throughout the entire organ.

SUMMARY

Dopan is effective in the treatment of twelve various experimental tumors. It is most effective in the treatment of fusiform sarcoma-45, Torashchansky's polymorphocellular sarcoma and carcinoma of the uterus in mice. The effect is moderate in case of Brown-Pearce tumors, polymorpho-cellular sarcoma M-1, Ehrlich's adenocarcinoma, Crocker's sarcoma and carcinoma of the mammary glands in mice. Little effect was observed in treating induced sarcoma in rats, leukosis of mice and cancer of the liver.

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PLANT EXTRACTS TESTED FOR CYTOTOXIC AND ANTITUMORIGENIC ACTION

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Research by massive experimentation is a method which can be used in the search for antitumorigenic preparations from plants due to the diversity of chemical compounds, the biological action of which is yet unknown, existing in plants. In a series of works [2, 3, 4], the order in which different representatives of the higher plants should be tested was determined by the definite pharmocopeian group with which they were connected.

Although there were no definite reasons for the choice of the material to be tested, in some cases, we were directed by various considerations concerning the type of biological action possessed by substances known to be or proposed to be contained in the given plant (for example, the presence of growth-inhibiting substances or of alkaloids with unknown biological action, etc).

EXPERIMENTAL METHODS

Only alcohol extracts were tested as, in this way, almost the whole mass of protein substances were excluded from the study. The alcohol extracts were prepared by the standard method: 50 g of dried plant was ground up and extracted with ethyl alcohol. The alcohol was completely evaporated in a vacuum (15 mm of residual pressure). The residue was dissolved in 100 ml of distilled water, filtered to remove undissolved matter and neutralized to pH 7.

Since most of the compounds with antitumorigenic properties also possessed cytotoxic properties, we first selected extracts according to their cytotoxic activity in vitro on tumorous cells of Ehrlich's ascites. We chose the method developed by V. A. Talyzina [1], already used in the laboratory of Prof. M. M. Maevsky for preliminary culling of antibiotics, to examine the cytotoxic effect in vitro. The test extract (neutralized to pH=7), in two dilutions, was added to fresh Ehrlich's ascites in a ratio of 1:1. The resulting mixture was kept in a refrigerator for 4 hours at a temperature of 4°, and then injected subcutaneously into 10 mice in a dose of 0.3 ml (each dilution was injected into 5 mice).

In this way, the entire process of the extract's action on the tumor cells proceeded in vitro, and only the result of this action was tested by transplanting the material processed with the test extract into the animal.

The control was the development of tumors on the opposite sides of the same mice caused by the simultaneous transplantation of an ascitesdiluted 1 : 1 with a physiological solution and also kept for 4 hours in the refrigerator.

The extract was considered to have cytotoxic properties if tumors either did not develop where the ascites processed with the test extract had been injected or developed a few days later than in the control.

Ninety alcohol extracts from 78 plants were examined, representing 39 different families. In some cases, extracts from different parts of the plant were tested separately.

Sixty-five of the plant extracts showed no cytotoxic effect in the experiments in vitro. The length of this article does not permit their enumeration.

When a cytotoxic effect was observed, the experiment was repeated once or twice.

Fifteen of the 90 extracts studied had, in some degree, cytotoxic properties. Our next task was to determine from in vivo experiments which of these extracts possessed an antitumorigenic action. However, during the in vitro experiments, we discovered that the cytotoxic effect of some of these extracts was evidently due to the fact that they contained a considerable amount of tannins, which, according to a previous test, do not have antitumorigenic properties and hinder the in vivo testing of an extract by causing extensive necroses to form at the injection place of the extract.

Therefore, the extracts in which a large tannin content had been determined were purified of the tannins by using skin powder before being tested again in vitro before the in vivo experiments.

To study the antitumorigenic properties of the selected extracts in vivo, we conducted the usual therapeutic experiments on mice with Ehrlich's tumor, implanted subcutaneously. Each extract was tested on 40 animals (20 in the experiment and 20 in the control). Treatment was begun on the 5th-6th day after implantation, when the tumors were of a measurable size (average diameter, 5-6 mm), and continued for 12 days. The extracts were injected subcutaneously in doses established earlier in experiments conducted on mice to determine the toxicity of the individual extracts.

At the end of the treatment period, the animals were killed, and the difference between the average weight of the tumors in the control and in the experiment was determined. This difference, expressed in percent of the average weight of the tumors in the control, served as the principal index of the antitumorigenic action of the extract.

Thirteen of the fifteen extracts which had been selected for their cytotoxic action were examined for antitumorigenic properties.

EXPERIMENTAL RESULTS

Positive results from both the in vitro and in vivo experiments coincided in 3 cases, i.e., the cytotoxic properties of the extract were coincidental with antitumorigenic properties (see table) these cases were the extracts from the above-ground portions of Anagallis and Datisca cannabina and from the fruit of the honey locust (Gleditsia triacanthos). However, the cytotoxic and antitumorigenic effects of the Anagallis extract were weakly expressed; treatment with this extract only inhibited growth in Ehrlich's tumor 30% and the extract was highly toxic.

The extract from the fruits of the Kirghiz honey locust had a stronger effect. There were many tannins contained in the native extract, which were removed before therapeutical use of the extract, but the second in vitro test showed that the extract retained its cytotoxic properties after the removal of the tannins. This extract inhibited the growth of Ehrlich's tumor 63%. One must mention, however, that the extract was rather toxic. By the end of the course of treatment, the experimental mice had lost weight considerably, and when they were dissected, a series of dystrophic changes were found in the liver and spleen. The Datisca cannabina extract (Gissar) had approximately the same effect.

Examination of the extract made from the rind of the Tyan-Shan Hippophae rhamnoides first gave us the impression that the cytotoxic effect of this extract was coincidental with its antitumorigenic properties. This impression proved to be false. The therapeutic experiment was first set up with the native (containing tannins) extract, greatly diluted to prevent necroses. The result obtained was an antitumorigenic effect, inhibiting the growth of Ehrlich's tumor 51%. When the extract was tested in vitro again, after the tannins had been removed, we found that its cytotoxic properties had been removed with the tannins. However, the antitumorigenic effect of treatment with the purified extract increased to 60.6% (as the concentration of the solution increased).

Results from In Vitro and In Vivo Tests of Alcohol Extracts

Family: genus and species of plant	Part of plant extracted	Result of test	
		in vitro	in vivo
Compositae			
Acroptilon picris	Above-ground portion	+	—
Artemisia Tournefortiana	The same	+	—
Saussurea sp.	" "	±	—
Centaurea balsamita	" "	+	Not studied
Oleaster			
Hippophaë rhamnoides:	" "		
Native extract after precipitation of tannins		+	+
			+
Rosaceae			
Padus racemosa:	" "		
Native extract after precipitation of tannins		+	Not studied
			—
Nymphaeaceae			
Nuphar luteum	Roots	+	—
Leguminosae			
Gleditsia triacanthos:	Fruit		
Native extract after precipitation of tannins		+	Not studied
		+	+
Equisetaceae			
Equisetum hellocharis	Above-ground portion	+	—
Primulaceae			
Anagallis sp.	The same	+	±
Umbelliferae			
Angelica brevicaulis	" "	+	—
Cucurbitaceae			
Cucumis melo	" "	+	—
Zygophyllaceae			
Peganum harmala	" "	+	—
Aceraceae			
Acer negundo	" "	+	Not studied
Datiscaceae			
Datisca cannabina	" "	+	+

Note: Symbols for in vitro experiments designate: + presence of cytotoxic effect (tumors did not dvelop); ± weak effect (all tumors in the experiment at least twice as small as in control on the 8th day after implantation); - no effect. For in vivo experiments: + clear positive effect (tumor growth inhibited more than 50% as compared with the control); ± weak positive effect (tumor growth inhibited 30-50%); -negative effect (tumor growth inhibited less than 30%).

Therefore, in the first in vitro experiments, the cytotoxic effect was caused by the presence of the tannins, but the antitumorigenic effect is connected with the presence of other substances which have not yet been investigated.

Nine of the extracts which manifested a cytotoxic effect in vitro did not show antitumorigenic activity in the therapeutic experiments. One must mention, however, that in some cases this may have been due to the rapid combination of the active substances directly on the place of injection into the body and to the insufficient concentration of these substances in a given extract. Therefore, some of the experiments which were done with less than the maximum therapeutic dosage bear repeating with more concentrated extracts.

We shall further investigate the three plants which had marked antitumorigenic properties in order to isolate the source of antitumorigenic activity and determine its chemical nature.

SUMMARY

90 extracts of 39 different plants were investigated, regarding their cytotoxic effect on the cells of Ehrlich's ascites. This effect was found to be present in 15. Out of this number, 13 were tested in experimental treatment of Ehrlich's tumor. The extracts of Hippophae rhamnoides, Datisca cannabina and Gleditsia triacanthos had pronounced retarding effect on the growth of this tumor.

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ON THE IMMUNITY OF RABBITS IN WHICH HIGH-INTENSITY ULTRASONIC WAVES HAVE CAUSED RESOLUTION OF THE BROWN-PEARCE TUMOR

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Retained immunity is known to occur after spontaneous tumor resolution in animals, and subsequent reimplantations of the tumor are not successful [1, 2, 5, 6, 7]. However, every tumor resolution does not create immunity in animals. For example, the resolution of implanted tumors which can be brought about by the action of chemical and hormonal preparations and roentgen rays do not make the animal immune to reimplantations of the tumor.

It has already been established that the action of highly intense ultrasonic waves 60-80% of the cases, causes the resolution of Brown-Pearce tumors in rabbits [4]. In many cases, the disintegration and resolution of metastases which had not been treated by the sound waves were observed.

Are rabbits in which the action of high-intensity ultrasonic waves has caused the regression of tumors then immune? The purpose of this work was to investigate this question.

EXPERIMENTAL METHODS

The investigation was done with 34 rabbits in which Brown-Pearce tumors had been resolved due to the action of high-intensity ultrasonic waves. The rabbits were treated with the sound by G. D. Andreevsky's with the aid of the high-powered, ultra-acoustic apparatus designed by A. K. Burov [3]. We used a frequency of 750-1500 kilocycles, a power of 34-150 w /cm^2 and an exposure of 1 $^1/_2$-75 seconds.

EXPERIMENTAL RESULTS

Resolution of the original tumor, implanted in a muscle or testicle of the rabbits, occurred at different intervals after the action of the high-intensity ultrasonic waves (Table 1).

TABLE 1

Time of Brown-Pearce Tumor Resolution in Rabbits After Sound Treatment

Time of tumor resolution after sound treatment (in months)	1	2	3	4	5	6	Total No. of rabbits
Number of rabbits with resolved tumor	3	17	7	2	2	3	34

* Deceased.

In most of the rabbits, as Table 1 shows, the tumor was resolved 2-3 months after the sound treatment; however, in some animals, the tumor was resolved during the first month, while, in others, the resolution process lasted as long as 6 months. The tumor usually became slightly larger before its resolution.

The rabbits' resistance was tested by one or two reinoculations of the Brown-Pearce tumor. The tumor was implanted once in all of the rabbits (34). The tumor was implanted twice in 13 of the rabbits. The tumors were reimplanted in various ways: in the testicle, intramuscularly and intraperitoneally. In order to prove the implantation, healthy rabbits (the control) were injected with the tumorous tissue. The tumorous suspension was injected in a dose of 1 $\frac{1}{2}$ - 2 ml in a dilution of 1:3 into both the experimental and control animals. The rabbits were observed for 2 years and carefully examined daily, with a detailed pathologico-anatomical examination at the end of the 2-year period.

The tumors were reimplanted at various intervals after the resolution of the sound-treated tumors (Table 2).

TABLE 2

Time After Resolution of Sound-Treated Tumor at Which Brown-Pearce Tumor Was Reimplanted in Rabbits

Time of reimplantations (in months)	1	2	3	4	5	6	7	8	9	10	11	12	Total No. of rabbits
Number of rabbits with one reimplantation of tumor	3	2	4	9	9	2			4			1	34
Number of rabbits with two reimplantations of tumor						2	2	1		1	6	1	13

As Table 2 shows, in most of the rabbits the tumor was reimplanted once, 4-5 months after the resolution of the original, sound-treated tumor. The interval between the first and second implantations was 5-7 months.

Our observations showed that in the 34 rabbits with the Brown-Pearce tumors which had been resolved after sound treatment, the reimplantation of the tumor, which was done at intervals varying from one month to one year after the resolution of the original tumor, was not successful. The tumor was not implanted by either the single or the double injection of the tumorous suspension (Table 3). This could be especially clearly observed in the rabbits with the tumor implanted in the testicle, where, by palpation, it was easy to trace the implantation of the tumorous suspension. With all the implantation methods we used (intramuscular, intraperitoneal, intratesticular), we could find neither necroses nor cicatrizations in either the place of implantation or the locations of the possible metastases (lymph nodes, internal organs) by pathologico-anatomical examination. The injection of the tumorous suspension into the healthy rabbits resulted in implantation in 100% of the cases and in the subsequent development of the tumorous process (12 out of 14 rabbits died from total metastasization), see Table 3.

TABLE 3

Results of Tumor Reimplantations

Animal group	Implantation	No. of rabbits	Tumor not implanted	Rabbits which died from metastases
Experimental	Once	34	34	0
	Twice	13	13	6
Control	Once	14	0	12

The work carried out allows the conclusion that the resolution of a Brown-Pearce tumor in rabbits after short exposure to high-intensity ultrasonic waves creates a lasting immunity in the animals to reimplantations of the rumor.

SUMMARY

The problem of whether rabbits with a tumor resolved by ultrasonic waves would develop resistance to new inoculations was studied.

Reimplantations of tumors into rabbits which had an implanted tumor resolved a month or a year previously under the action of ultrasonic waves were not successful.

We may draw the conclusion that resolution of a Brown-Pearce tumor caused by ultrasonic sound waves makes rabbits resistant to new inoculations.

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PROTRACTED HETEROTRANSPLANTATION OF THE MOUSE TUMOR TO RATS

ARTICLE 1: A STUDY OF THE BIOLOGICAL PROPERTIES OF MOUSE ASCITIC ADENOCARCINOMA SERIALLY TRANSPLANTED TO RATS

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The importance of tumor heterotransplantation and the difficulties of accomplishing it are well-known. As Ehrlich [8] has already established, when a mouse carcinoma is subcutaneously implanted in rats, the tumor grows for a short time (up to 8 days), but then is resolved. In some studies [7, 10], a rather long preservation of the heterotransplanted Ehrlich's mouse tumor was obtained, but only in rats of the Hungarian strain. Tumor growth in a different type of host has been protracted by shortening the intervals between implantations of the heterogeneous carcinoma [1, 6], but even in this way, prolonged and steady development of heterotransplanted tumors could not be maintained for long. There are interesting works on the long preservation of tumors heterotransplanted from mice to newborn rats [9].

The majority of authors [3, 4, 6, 9, 10] mention that certain biological properties of mouse carcinoma are changed by heterotransplantation. However, there is as yet no definitive explanation of this question.

Our purpose was to obtain the prolonged development of a mouse ascitic cancer tumor heterotransplanted to rats and to study the immunobiological properties of the tumor during the implantations.

EXPERIMENTAL METHODS

Three to five-day-old rats were intra-abdominally inoculated with a dose of 0.2 ml of Ehrlich's mouse adenocarcinoma (ascitic type). A considerable increase in the bulk of the animals was observed in the rats on the 3rd-6th day after implantation, and the rats died on the 6th-8th day. Material for subsequent implantations was obtained in the usual way under sterile conditions and bacteriologically inspected; at least 20 rats weighing from 6 to 9 g were used for each implantation (passage). When the increased volume of the rats' stomachs had reached considerable proportions, which usually occurred on the 3rd-6th day of the implantation, all the rats were injected with 1 ml of a physiological solution (0.85% NaCl) with penicillin (about 10,000 units); then, 2 ml or more of the ascitic fluid, thus diluted, was carefully pumped into a syringe. The ascitic exudate from the rats, even when diluted, was a rather dense suspension of pink or grey (depending on the erythrocyte content) tumorous cells. The material obtained was injected into the celiac cavity of normal rats in a dose of 0.2-0.4 ml.

The implantations of the mouse ascitic cancer in the 3-5-day old rats reached a frequency of 100%. In the last passages, the amount of tumorous cells per 1 ml of ascitic fluid fluctuated, although the amount was still rather considerable. It was 40 million cells in the 92nd passage and 66 million in the 108th passage, whereas, in ordinary mouse ascitic cancer, the amount of cancerous cells as a rule fluctuates between 99 and 120 million.

The cells were counted in a chamber with a Goryev's grid. The development of ascitic cancer in the

celiac cavity of the rats was also histologically proven. Smears of the ascitic exudate from the rats were fixed for one hour in a mixture of alcohol-ether and then stained with hematoxylin-eosin.

Five to eight white male mice weighing from 18-22 g and intra-abdominally inoculated with the tumor were used as the biological control for each passage.

We inoculated 2-week old or older rats and mice intra-abdominally with the passage cancer as an additional test to determine the virulency of the tumor in the passages. In these experiments, the implantation dose of ascitic cancer was 0.2-0.4 ml per 8 g of weight for the rats and 0.1-0.2 ml for the mice.

A total of 4,000 rats of various ages and 2,000 mice were experimented upon.

In this work, we shall present the general results of the observation from all 400 experiments.

EXPERIMENTAL RESULTS

The first successful experiments transplanting Ehrlich's ascitic mouse cancer to the celiac cavity of rats were done in May-June 1954. At that time, we succeeded in transplanting the mouse tumor to rats for five passages with no decrease in the growth intensity of the tumor when it was reversely transplanted to mice. Further transplantations were impossible, however, due to the death of the experimental rats.

In April, 1955, we again transplanted Ehrlich's ascitic mouse cancer to rats intraperitoneally. This time, in view of the real danger of losing the strain, we conducted 2-3 parallel series of transplantations, using 15-30 animals, aged from 3-6 days, for each passage. More than 100 passages have now been made in the rats.

Twice, during 16 months of transplanting mouse tumors to rats, we were obliged to reimplant the tumor in the mice (after 30 and 73 passages) before we could conduct further passages to the rats.

These reverse passages to the mice were not reflected in the properties or behavior of the strain, as subsequent transplantations showed. The tumor grew intensively in the celiac cavity of the rats, which was indicated by the increase in the volume of the ascitic fluid and by the numerous mitotic forms in tumor cells.

We were very interested to find whether the immunobiological properties of mouse carcinoma changed after such prolonged heterogeneous transplantations of the tumor. The results obtained from our examination of the antigenic properties of mouse carcinoma cells after transplantation to rats will be presented in the next article. Certain changes in the biological properties were observed as follows.

The external aspect of the ascitic exudate changed gradually during the process of transplanting mouse cancer to 3-5-day-old rats; after 52-58 passages, a more fluid ascitic exudate, grey in color, in which white flakes occurred rather frequently (conglomerations of cancerous cells), began to replace the thicker, pink or cherry-colored exudate. In addition, when the rats were dissected, a thin, whitish film began to appear at about the same time on the organs of the celiac cavity (liver, spleen), which also consisted of tumorous cell conglomerations. However, in some of the rats, the ascitic exudate which developed was different, rather thick and grey without the flakes or films; this exudate was also used for further implantations. During this same period, we increased the inoculation dose from 0.2 to 0.4 ml and shortened the interval between implantations from 5-6 to 3-4 days. Tumorous cells of Ehrlich's mouse carcinoma were constantly found in the smears of the rat ascitic exudate.

When the ascitic cancer was transplanted back to the mice, we observed that 1-40 generations caused the amount of exudate to increase (to 9-10 or more ml) and that the mouse died, on the average, 10 to 14 days after the implantation. After 41-45 generations, the control mice, on the average, died sooner (on the 8th-12th day), but the accumulation of the ascitic cancer exudate was very slight (0.5-1.5 ml and less than 3 ml). The volume of cancerous exudate was also observed to decrease in further passages of the heterotransplanted tumor to mice; when the number of passages to rats (8, 20, 30, 44, 55 generations) increased, the amount of tumor implantations in mice (16,11, 5, 4, 3 passages) decreased. In the last passages to the mice, no ascitic exudate was observed to develop in the mice, although the animals died.

Implantations of ascitic cancer to the celiac cavity of rats 2 weeks old or more were observed to first increase and subsequently to decrease the virulency of the tumor to more adult rats.

In contrast to the mice, the rats all survived the last passages, and the tumor was resolved.

Therefore, a new method of protracted mouse tumor heterotransplantation to rats has been developed

which permits more than 110 implantations of Ehrlich's ascitic carcinoma to be done within the celiac cavity of young rats (chiefly, of rats from 3-5 days old).

The heterotransplantate grew and developed like the original mouse carcinoma in both mice and week-old rats, retaining the basic morphology of the original Ehrlich's strain. However, protracted existence in heterogeneous surroundings caused some change in certain biological properties of the mouse ascitic carcinoma. For example, heterotransplantation gradually caused the tumor to lose one of the main features of mouse carcinoma, i.e., the ability to form a large amount of ascitic fluid with the implantation of tumorous cells in the celiac cavity of mice; the tumor became less virulent in relation to rats aged 2 weeks or more (after the 50th generation) and its rate of growth decreased in both rats and mice; the outward appearance of the ascitic cancer exudate in the young rats changed (after 52-58 passages). Therefore, the heterogeneous strain of the tumor cannot be considered identical to the original Ehrlich's ascitic mouse carcinoma.

SUMMARY

Over 100 intra-abdominal heterotransplantations of Ehrlich's ascitic adenocarcinona to 3-5-day old rats were performed. Although the essential nature of the initial ascitic cancer was preserved for 16 months, some of its biological effects on mice and rats were altered, e.g., the mice did not form a large amount of ascitic fluid. In rats two weeks old and older, the growth rate of the heterogeneous implantations decreased from that of the original virulent mouse tumor.

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ON THE POSSIBILITY OF TRANSPLANTING LYMPHOBLASTOMA AT DIFFERENT INTERVALS AFTER THE HOST'S DEATH

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It is known that experimental tumors and leukosis can be reproduced by using implantations of blastomatous or tumorous tissue from animals just killed [1, 2, 3, 4, 5, 6, 7 and others]. We could find no indication in the literature as to the time limit for positive results from the given transplantations. This question is important for two reasons: first, because it is not always possible to obtain tissue for transplantation immediately after the patient's death, and second, because it would be interesting to know how long after the death of the host the blastomatous and leukotic cells retain their vitality.

EXPERIMENTAL METHODS

We produced an implantation of LIO lymphoblastomatous tissue by means of approximately 80 passages of the tissue to a highly leukotic strain (Afb) of white mice. Five mice with a more or less generalized lymphoblastoma were used as the donors. The number of leukocytes in the peripheral blood of the animals was 32,200, 35,020, 36,400, 50,800 and 60,000 per 1 mm^3. We found lymphoblasts (up to 19%), prolymphocytes and single normoblasts in the blood smears.

The animals were killed by ether narcosis; their bodies were kept at room temperature (about + 18°), and several transplantations were made from each animal. Under sterile conditions, pieces of tissue were taken from the enlarged spleen, the lymph nodes and subcutaneous tumorous growths of the animal directly after its death and then after 6, 12, 24, 30, 48, 60 and 75 hours. A suspension was prepared from the tissue pieces in a physiological solution; 75,000-100,000 cells per 1 mm^3 were contained in the suspension, which was then injected either subcutaneously or intra-abdominally into healthy white mice of the Afb. strain, 1-1 ½ months old, in a dose of 0.3 cc.

At the same intervals after the death of the animals, pieces were also resected from the organs and tissues which had been used in the preparation of the suspension and also from the liver, kidneys and lungs to be used for microscopic examination, and impression preparations were made from the lymphoblastomatous nodes. The dried pieces were fixed in 10% neutral formalin and embedded in celloidin. The sections were stained with hematoxylin-eosin by Van Gieson's method and were impregnated with silver nitrate according to Gomori.

The transplantation suspension was proven sterile in selective bacteriological examinations.

The blood of the mouse recipients was examined at different intervals after the beginning of the experiment (after 7, 10, 12 days, and every 5 days thereafter in longer experiments). After the death of the animals, a pathologico-anatomical autopsy was done, and the pieces of tissue were microscopically examined.

In all, 82 mice and 8 different periods after the death of the host were experimented with.

EXPERIMENTAL RESULTS

The experiments conducted showed that, to obtain positive implantations, one should consider the time limit of keeping the dead animals at room temperature to be 60 hours (see Table). There was apparently some technical error made in the experiments with lymphoblastoma transplantation 24 hours after the death of the donor mice, which explains the small number (2) of positive implantations. The number of successful implantations began to decrease in the 30-hour experiments. There were negative results after the 2-3 month observation period in the mice which had received material taken 75 hours after the death of the donor mice.

The first palpable development of the subcutaneously implanted lymphoblastoma in the animals appeared on the 7th-10th day; in experiments with transplantation tissue taken at longer intervals after the death of the donor (48-60 hours), the transplanted lymphoblastoma began to grow at slightly later dates, but the tumorous tissue then quickly spread, and the mice died at the same intervals as in the other experiments.

TABLE

Results of Lymphoblastoma Tissue Suspension Implantations

Time of transplantation after animals death (in hrs.)	No. of mice in experiment	No. of positive implantations	Time animals died after implantations (in days)
Immediately	12	11	17—30
6	8	7	17—41
12	8	8	14—37
24	8	2	14—37
30	10	7	15—33
48	15	8	22—35
60	10	4	19—28
75	11	—	—

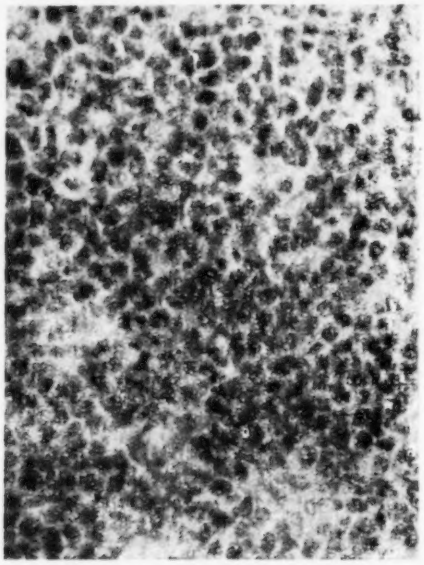

Figure 1. Histological structure of the original transplanted lymphoblastoma.

In 29 of the selectively examined recipient animals with a definite growth of tumorous tissue, the number of leukocytes found in the blood was from 30,000 to 65,000 per 1 mm^3, and there was a predominance of lymphoblasts, often in a condition of karyokinetic division. In 18 of the animals, the number of leukocytes was 11,000 to 26,000 per 1 mm^3, and monocytic cellular elements predominated in the blood.

When the donor animals were dissected, a generalized, subcutaneous lymphoblastoma with leukotic changes in the internal organs was found. In the recipient mice which died, in the subcutaneous cellular tissue at the place of implantation, there were tumorous nodes as large as 3-4 x 2 cm consisting of rather soft, pale pink, homogeneous tissue. Where the lymphoblastoma tissue suspension had been injected intra-abdominally, the same type of tumorous formations as appeared with the subcutaneous injection were found in the mesenteric roots and in the anterior mediastinum. The lymph nodes in these animals were enlarged 2-3 times; in the experiments with longer intervals after the death of the animal before the preparation of the lymphoblastomatous tissue suspension, the lymph nodes in the neck were especially large. The spleen was 3 times it normal size.

When the tumors in the donor and recipient mice were microscopically examined, typical lymphoblastoma was found: the tumor cells were homogeneous, large, with a round nucleus, did not stain intensely and had a small quantity of homogeneous protoplasm. The cells were closely packed and many instances of karyokinetic division were found among them (Fig. 1). Capillaries were found occasionally; the argyrophilic fibers were thin and sparse. Almost the entire tissue of the lymph nodes and spleen had been replaced with such tissue. There were small perivascular accumulations of lymphoblasts in the liver and, in some animals, in the kidneys and

peribronchially in the lung tissue. We found a large number of large and small lymphoblast cells with well-stained nuclei on the impression preparations.

Six to twelve hours after the death of the donor mice, microscopic examimination showed signs of post-humous decay in the liver and kidneys. There were no longer any dividing cells in the lymphoblastoma nodes; the nuclei of the lymphoblasts stained more intensely than formerly, and in some of the cellular elements, fragmentation of the nuclei was observed. These changes gradually intensified, and 48 hours after the death of the animal donors, nuclei of different sizes were observed in the lymphoblasts, many of them as large as lymphocytes and intensely stained; many of the cell nuclei disintegrated into large lumps. However, typical lymphoblasts were still found among the changed cellular elements (Fig. 2). The argyrophilic fibers were intensely impregnated with silver nitrate. Swollen hepatic parenchyma cells were observed and the nephritic epithelium, especially in the convoluted tubules, was swollen, with cloudy protoplasm and nuclei which were not always clearly stained.

Sixty hours after the death of the animals, the disintegration of the cell nuclei was sharply expressed in the peripheral sections of the tumorous nodes; in places, they were not stained at all. In the other sections, the lymphoblastoma tissue had greatly disintegrated; the cells were polymorphous with, for the most part, small nuclei, frequently angular in shape, diffusely and intensely stained; extremely numerous, small granules of chromatin were found, and typical lymphoblasts could still be seen, but not in every microscopic field (Fig. 3). The argyrophilic fibers were partially fused and in a state of fragmentation. Detritus from the decaying cells, granules of chromatin and occasionally comparatively well-preserved lymphoblasts were observed on the impression preparations. Decay was sharply expressed in the liver and kidney tissue — many cellular elements did not stain. Macroscopically, the changes caused by posthumous decay were very apparent, especially in the gastrointestinal tract.

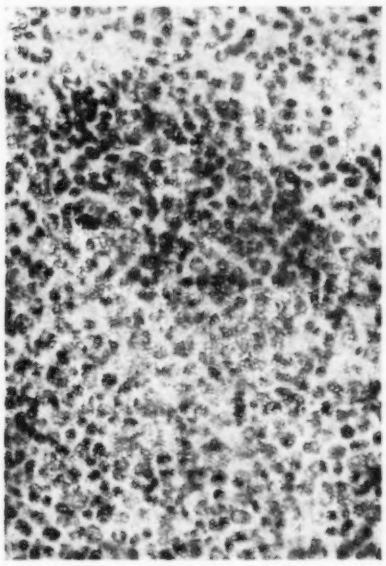

Figure 2. Histological structure of lymphoblastoma 48 hours after the animal's death.

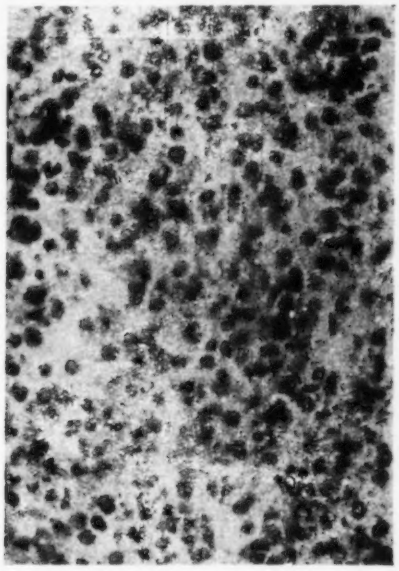

Figure 3. Histological structure of lymphoblastoma 60 hours after the animal's death.

Seventy-five hours after the animals' death, organic decay was acute. The cellular elements in the liver, kidneys and lungs did not stain. No preserved cells could be found in the tumorous nodes; the tissue consisted almost entirely of detritus with many small clumps of chromatin.

Generally, one can say that there was a parallelism between the number of positive lymphoblastoma implantations to the recipient mice and the histo-morphological picture of the transplant tissue at different times after the death of the donor animals.

From the experiments conducted, one can conclude that transplantations can succeed up to 60 hours after the death of the host animals, even when their bodies are kept at ordinary room temperature. At this time, most of the cellular elements of the transplant tissue are in a state of decay, and only individual lymphoblastic cellular elements are still preserved. These elements are apparently more resistant to the process of posthumous decay than the tissue of the liver and kidneys.

SUMMARY

Transplantation of cellular suspensions of lymphoblastoma, kept at 18°C is successful if performed not later than 60 hours after the host has died. The number of successful transplantations decline if performed 30 hours after the death of the host. No transplantation has ever been successful if made 75 hours after the host's death. Microscopic studies made of later transplantations revealed a large amount of detritus in the implanted tissue.

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EXPERIMENTAL BIOLOGY

A STUDY OF THE ANTIGENIC PROPERTIES OF THE CRYSTALLINE LENS IN WOLFFIAN REGENERATION

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One aspect of the study of the rules regulating the formative processes is the investigation of the minute changes in the protein composition of the developing tissues, which finally result, evidently, in a change in the morphological structure of these tissues. Since the change in tissue protein composition is attended by a change in the antigenic properties of the tissues, studying these properties can definitely help solve the problem regarding the mechanism of the formative processes.

It has recently been established that the tissues of the various animal and human organs particularly contain antigens specific to the given organ alone (organo-specific antigens). It has also been established that the various organo-specific antigens appear at definitely fixed stages of embryonal development [3, 4, 7].

The most generally accepted point of view is that the presence of an organo-specific antigen is connected with a definite function being fulfilled by the given organ [5, 6, 8].

However, in 1956, B. V. Konyukhov published a work presenting data showing that the organo-specific antigen of the crystalline lens could be found in the placode stage, i.e., at a period when the actual function of the crystalline lens does not exist.

There is a question which must be specifically settled in connection with this: do organo-specific antigenic properties depend on the fact that the given organ is carrying out a definite function, or are these properties dependent on the origin of the given tissue from a definite tissue source?

The purpose of our work was to solve this problem.

We used the phenomenon of so-called wolffian regeneration of the crystalline lens in our work.

When the crystalline lens is removed from a triton's eye, the triton is known to form a new crystalline lens, not in the usual way – from the ectodermal epithelium, but from the upper edge of the iris, i.e., from tissue derived from the neural tube. Therefore the crystalline lens formed by wolffian regeneration performs the same function as an ordinary crystalline lens, but has a different origin.

It was important to establish whether the tissue formed in wolffian regeneration of the crystalline lens would have the same antigenic properties as the tissue of an ordinary crystalline lens or whether these properties would differ according to the difference of tissue origin.

The solution of the question has not been described in the literature.

EXPERIMENTAL METHODS

The crystalline lens was removed from the right eye of tritons (Tr. taeniatus) under ether anesthesia. The tritons were fastened down in a small paraffin bath by fine needles in such a way that the right eye was uppermost. The cornea was carefully slit with a lanceolate knife and a glass rod, and the crystalline lens removed with finely pointed pincers.

The animals which had been operated upon were kept for 5 and for 15 days. After this, their iris tissues were removed and ground with a few drops of a physiological solution of NaCl. The resulting paste was used in the subsequent experiments.

To study the antigenic properties of the tissues forming from wolffian regeneration, we used the anaphylactic reaction, the use of which in determining the antigenic properties of embryonic tissues was demonstrated earlier by B. V. Konyukhov and R. F. Averkina [1, 2, 3].

Guinea pigs were sensitized subcutaneously with an emulsion from the tissue of 20 irises, isolated 5 and 15 days after the removal of the crystalline lens. Guinea pigs sensitized by an emulsion from the same quantity of iris tissues, isolated from the same species of animal in which the eyes had not been operated upon, were used as the control.

On the 30th day after sensitization, all the animals were injected intravenously with 60 mg of a water-salt extract from the crystalline lens tissue of a frog (Rana ridibunda).

We chose the crystalline lens of the frog, rather than of the triton, in order to avoid the possibility of a reaction fromspecies-specific antigens.

The extract for the reacting injection was prepared as follows. The crystalline lenses were carefully ground in a porcelain mortar, and 9 volumes of a physiological solution of NaCl were added. The resulting emulsion was shaken for 20 minutes and then centrifuged for 20 minutes at a speed of 2,000 revolutions per minute.

The degree to which the anaphylactic reaction was expressed was determined by the usual method [2].

EXPERIMENTAL RESULTS

The results of the experiments described are given in the table.

Anaphylactic Reaction in Guinea Pigs, Sensitized by Iris Tissues, in Response to Reacting Injection of the Crystalline Lens Extract From a Frog

No. of guinea pig	Sensitization		Reaction injection		Reaction
	Antigen	Dose (irises)	Antigen	Dose (in mg)	
1067	Iris on the 5th day after removal of the crystalline lens	20	Crystalline lens	60	—
1034	The same	20	The same	60	—
1363	The same	20	» »	60	—
1926	Iris on the 15th day after removal of the crystalline lens	20	» »	60	+
1943	The same	20	» »	60	+ + +
1906	The same	20	» »	60	+
1312	Iris from eyes not operated upon	20	» »	60	—
1313	The same	20	» »	60	—
1792	The same	20	» »	60	—

As the table shows, the reaction to the reacting injection of the antigen from the tissues of the frog crystalline lens tissue was negative in all of the guinea pigs which had been sensitized by the emulsion from the tissues of irises taken from the tritons on the 5th day after removal of the crystalline lens. All the guinea pigs sensitized by the emulsion from the iris tissue taken from the tritons on the 15th day after removal of the crystalline lens, however, gave a positive anaphylactic reaction in response to the reacting injection of the antigen.

The reaction was negative in the control animals.

Figure 1. Early stage of crystalline lens formation in triton. Edge of iris breaking up, beginning of iris depigmentation. The crystalline lens antigen was not observed at this stage (5th day of experiment).

Therefore, the organo-specific crystalline lens antigen was discovered in the iris tissue on the 15th day after the removal of the crystalline lens. This antigen was not found on the 5th day.

On the 5th day after the crystalline lens had been removed, the crystalline lens formation process had just begun. At this stage, the edge of the iris was breaking up and beginning to become depigmented (Fig. 1). By the 15th day, there was already a well-formed, although not yet completely separated, crystalline lens on the upper edge of the iris (Fig. 2). The crystalline lens which had formed from the iris was exactly like the ordinary crystalline lens in its histological structure.

Therefore, the specific, crystalline lens antigen could be observed in a crystalline lens, when sufficiently formed, which was derived from the iris. Although, due to the reasons given earlier, only one organ was used in the work, i.e., the crystalline lens, our data allow the propostion that organs fulfilling the same function and having the same histological structure possess similar antigenic properties, although their origin may not be the same.

Figure 2. Crystalline lens which has formed from the iris. The antigens specific to the crystalline lens were clearly evident at this stage (15th day of experiment).

Of course, the fact that the same antigens are present in the ordinary crystalline lens and in the regenerated crystalline lens does not exclude the possibility that there are also different antigens in these tissues, which owe their presence to the difference in the origin of the tissues. However, we are now conducting the special study which is needed to solve this problem.

SUMMARY

Experiments on tritons have shown that on the 15th day of wollfian regeneration of the crystalline lens normally extirpated from the iris, an antigen specific for the ctystalline lens appears in that tissue. No antigen could be detected at earlier stages of regeneration (on the fifth day). It is supposed that organs of the same function and histologic structure, no matter of what origin they are, have identical antigenic properties.

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A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF TISSUES IN ANIMALS OF VARIOUS SPECIES AT DIFFERENT STAGES OF DEVELOPMENT

ARTICLE II: A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF HEART TISSUES FROM THE CHICKEN, GRASS SNAKE AND FROG

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The purpose of our research was to study how antigenic specificity forms in animal tissues and also to determine whether recurrence of the antigenic properties of tissues in less highly organized animals occurs during the process of individual development.

Earlier [1], we reported our data from a comparative study of the antigenic properties of muscle tissue from caudate and noncaudate amphibians. It was established that the muscular tissue of the tadpole has antigenic properties that differ considerably from those of the frog's muscular tissue; this difference is constituted in the fact that there are antigens present in the tadpole muscle tissue which are lacking in that of the frog.

It was also established that there is less difference between the antigenic properties of tadpole and triton muscle tissue than there is between those of frog and triton muscle tissue.

In this work, the antigenic properties of heart tissues from chicken embryos at various stages of development (5, 6, 8, 12, 14 and 18 days of incubation) and the heart of an adult chicken are compared with the antigenic properties of heart tissues from the grass snake and the frog.

The complement fixation reaction was used in the work, and set up according to the usual method.

EXPERIMENTAL METHODS

First, immune serum was obtained against water-salt extracts from the heart tissues of the chicken, grass snake and frog. Water-salt extracts from the heart tissues of chicken embryos incubated 5, 6, 8, 12, 14 and 18 days and from that of an adult chicken were used as antigens. The method of preparing the extract and of obtaining the immune serum was described in the first article [1]. The complement fixation reaction was set up with the serum and antigens obtained from the heart tissues of the chicken embryos and of the adult chicken. Before the reaction was set up, the amount of protein in the antigens was determined by the Kjeldahl method. After this, the amount of protein in all of the antigens was equalized by diluting the more concentrated antigens with a physiological solution.

The results of the experiments are given in Tables 1 and 2, each of which summarizes the data obtained from 60 reactions. •

• The average amount of the experiments was computed between the three analogous immune serums and all the antigens from the chicken embryo hearts.

EXPERIMENTAL RESULTS

The serum against the chicken heart tissue reacted with the antigen from the heart tissues of chicken embryos incubated 4 and 6 days in a dilution of 1:80. The reaction with the antigen from the heart tissue of the chicken embryo incubated 6 days was slightly more intense. The same serum reacted with the antigen from the heart tissue of 8- and 12-day incubation chicken embryos in a dilution of 1:160. One must note that the reaction of the serum against the chicken heart tissue was slightly more marked with the antigen from the heart tissues of the chicken embryo incubated 12 days. The serum against the chicken heart tissue reacted with the antigen from the tissues of chicken embryos incubated 14 and 18 days in a dilution of 1:320, and with the antigen from the adult chicken heart tissue, in a dilution of 1:640 (Table 1).

TABLE 1

Titers and Intensity of Complement Fixation Reaction of the Serum Against the Chicken Heart Tissue with the Antigens From Hearts of Chicken Embryos at Various Stages of Development

Serum dilution	Antigens						
	Duration of chicken embryo incubation (in days)						Adult chicken
	4	6	8	12	14	18	
1:20	±	+++	++	++++	+±	+++	++
1:40	+++	+++	±	++++	+++	++	±
1:80	+	++	±	+±	+++	++	++
1:160	–	–	+	±	++	+±	±
1:320	–	–	–	–	+	±	+
1:640	–	–	–	–	–	–	+

Comparing the titers and reaction intensity of the serum against the chicken heart tissue with all the antigens from the chicken embryo heart tissues used in the experiment showed that the greatest difference in antigenic properties was between the heart of the adult chicken and that of the chicken embryos incubated 4 and 6 days. Then, as the chicken embryo became more developed, this difference in heart tissue antigenic properties gradually decreased. Therefore, the results of this experiment confirmed the data of other researchers [2 and others].

Table 2 shows that the serum against the heart tissue of the grass snake reacted with the antigen from the heart tissues of the chicken embryos incubated 5 and 6 days in a dilution of 1:320, with the antigen from the heart tissue of the chicken embryo incubated 8 days, in a dilution of 1:160, with that from the heart tissue of the embryos incubated 12 and 14 days, in a dilution of 1:80, and with the antigens from the heart tissue of an 18-day incubated embryo and from the adult chicken heart, in a dilution of 1:40. Comparing the titers and reaction intensity of the serum against the grass snake heart tissue with the antigens from the chicken embryo heart tissue and from the adult chicken heart showed that the antigenic properties of the hearts from the chicken embryos incubated 5 and 6 days were more similar to those of the grass snake heart. Then, in the same way, as the chicken embryo developed, this similarity decreased.

In the next series of experiments, results were obtained from complement fixation reactions between the serum against the frog heart and the antigens from the heart tissues of chicken embryos at the same stages of incubation as were used in the reaction with the serum against the grass snake heart. The serum against the frog heart tissue reacted with the antigen from the heart tissues of chicken embryos incubated 5, 6 and 8 days in a dilution of 1:160, with the antigen from embryos incubated 12 and 14 days, in a dilution of 1:80 and with the antigen from the adult chicken heart tissue, in a dilution of 1:40 (Table 3).

Comparing the titers of the reaction of the serum against the frog heart tissue with the antigens from the chicken embryo heart tissues and with the antigen from the adult chicken heart showed that the antigenic properties of the heart tissue from chicken embryos at the earlier stages of incubation were more similar to those of the frog heart tissue than were those of the heart tissue from embryos at later stages of development.

TABLE 2

Titers and Intensity of Complement Fixation Reaction Between Serum Against Heart of Grass Snake and Antigens From Hearts of Chicken Embryos at Various Stages of Development

Serum dilution	Antigens						
	Duration of chicken embryo incubation (in days)						Adult chicken
	5	6	8	12	14	18	
1 : 20	++++	+++	+++	+++	+++	++	++
1 : 40	++++	++ +	+++	++	++	+	+
1 : 80	+++	+ +	++	+	+		
1 : 160	++	++	+				
1 : 320	+	+					
1 : 640							

TABLE 3

Titers and Intensity of Complement Fixation Reaction Between Serum Against Frog Heart and Antigens From Hearts of Chicken Embryos at Various Stages of Development

Serum dilution	Antigens					
	Duration of chickenembryo incubation (in days)					Adult chicken
	4	6	8	12	14	
1 : 20	+++	+++	+++	+++	+++	+++
1 : 40	+++	+++	+++	+	+	+
1 : 80	+++	+	+			
1 : 160	+					
1 : 320						
1 : 640						

Therefore, the results of our experiments showed that, during the development process of the chicken embryo, the antigenic properties of its heart tissue become increasingly more similar to those of an adult chicken's heart. The antigenic properties of heart tissue from chicken embryos incubated 5 and 6 days are more similar to those of the grass snake and frog hearts. As the chicken embryo develops, this similarity decreases.

Based on the marked antigenic similarity of the heart tissues from chicken embryos at the earlier stages of development with the heart tissues of the grass snake and frog, one can propose that, at these stages, the antigenic properties of the bird's ancestors, which were somewhat similar to contemporary reptiles and amphibians, are reflected.

SUMMARY

Antigenic properties of the heart tissues of chicken embryo of 5, 6, 12, 14, 18 days, of mature chickens, reptiles (Naotrix natrix) and amphibia (Rana ridibunda) were comparatively studied by the complement fixation test. With the development of the chicken embryo antigenic properties of the heart tissues approach more and more those of the mature chicken. In the more early embryonic periods they resemble those of the grass snake and frog. With the development of the chicken embryo this similarity becomes less and less pronounced.

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AN ONTOGENETIC STUDY OF THE ANTIGENIC PROPERTIES OF ANIMAL TISSUES AND ORGANS

COMMUNICATION 4. AN EMBRYOGENETIC STUDY OF THE SPECIES- AND ORGANOSPECIFICITY OF A CHICKEN'S HEART

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The data from works studying the antigenic properties of the heart in embryogenesis are extremely contradictory. For example, Ebert [4], starting from results obtained from the precipitin reaction, stated that the organospecific antigens of the heart were present in chicken embryo tissues at very early stages of the embryo's development (primitive streak stage) and that they were the same as the antigens of the brain. However, the experimental results which Ebert obtained in this same work by the use of the tissue culture method contradict the above data. No heart pulsations developed in the embryos cultured on a medium to which anti-chicken heart serum had been added. Heart pulsations, however, developed in embryos cultured on a medium containing an anti-chicken brain immune serum. Based on the results which Ebert received from the precipitin reaction [4], one would expect that, due to the antigenic similarity of the heart and brain at the early stages of embryogenesis, the anti-brain immune serums would inhibit the development of the heart.

Later, Ebert [5] presented data indicating that, in his opinion, this general "cardio-cerebral" antigen complex was only found in chicken brain tissues on the 18th day of incubation.

Recently, the results of researches [6, 7] making an immunological study of the contraction proteins (myosin and actin) in a developing chicken's heart have been published. The authors confirm that the myosin and actin of a definitive chicken's heart may be found as early as the primitive streak and cephalization stages; myosin synthesis is begun long before the cellular elements which comprise the foundation of the heart begin to form (in embryos of 12-13-hour incubation). However, according to the data of Johnsen and Leone [8], definitive chicken's heart actomyosin does not appear in the embryo until after it has been incubated for 40 hours.

Therefore, the problem of the formation of the heart antigenic structure is still unsolved. This makes it difficult to understand the ontogenetic development of the antigenic properties of animal tissues.

The purpose of this work was to further investigate the formation process of the antigenic structure of the heart.

EXPERIMENTAL METHODS

We used the anaphylactic reaction with desensitization and the ring-precipitin reaction. We have already described how these reactions were conducted [1, 2]. The serums against the heart tissues of an adult chicken which were used in the ring-precipitin reaction were absorbed by the hepatic cells of the chicken

according to the method proposed by P. N. Kosyakov, V. S. Korosteleva and N. I. Kuznetsova [3].

The first series of experiments examined the antigenic species-specificity of the developing heart tissues. Guinea pigs were sensitized subcutaneously with suspensions of heart tissues from chicken embryos (Russian White species) a different stages of incubation (3, 4, 6, 8, 10, 12, 16 and 19 days), and also from an adult chicken. The heart tissues were carefully washed several times in a physiological solution to remove the blood. The washing water was examined for serous protein content by the ring-precipitin reaction. When the last specimens of washing water gave no positive reaction with the immune serum against the adult chicken serum (titer of serum according to antigen dilution, 1:50,000), the tissues were used for sensitization. On the 21st day after the sensitizing injection, all the animals were given a reacting injection of chicken serum either intravenously or intraperitoneally, depending on the dose. Large doses of the serum (500 mg) were injected intraperitoneally. The same antigen was injected intravenously into 3 nonsensitized animals (technical control).

The second series of experiments was conducted to determine the point at which the organospecific antigens of the definitive heart appeared. Animals were used which had been desensitized to species-specific antigens by an injection of serum from an adult chicken and then tested to confirm the completeness of their desensitization (intravenous injection of 300 mg of serum). Two hours after the test, all the animals were given intravenously a reacting injection of a water-salt extract from adult chicken heart tissues. The extract was also injected into 3 nonsensitized guinea pigs (technical control).

The extract was obtained in the following manner. Heart tissues, washed free of blood, were gound in a homogenizer with 4 volumes of a physiological solution. The resulting suspension was centrifuged for 20 minutes at a speed of 3,000 revolutions per minute.

EXPERIMENTAL RESULTS

All the sensitized guinea pigs responded to the chicken serum injection by a positive anaphylactic reaction (Table 1). The reaction was somewhat more pronounced in the guinea pigs sensitized by the suspensions of heart tissue from chicken embryos at the later developmental stages, in some cases, so pronounced that when the antigen was injected intravenously, the animal died. In order to preserve the animals, the injections were done intraperitoneally. There was no reaction observed in the control animals.

Therefore, at all the developmental stages studied, the heart tissues containedspecies-specific antigens. This antigenic species-specificity evidently increased as the embryo developed.

The experimental results of the second series are also given in Table 1. No positive anaphylactic reaction was observed in the guinea pigs sensitized with suspensions of heart tissue from 3 and 4-day chicken embryos. Nor was a positive reaction observed in the control animals. In the animals sensitized with suspensions of heart tissue from embryos incubated 6, 8, 10, 12, 16 and 19 days and from an adult chicken, however, a positive anaphlyactic reaction was observed in response to the injection of a water-salt extract from the tissues of a definitive chicken heart.

Therefore, the results of this experimental series showed that the heart tissue of embryos incubated 6, 8, 10, 12, 16 and 19 days contains organospecific antigens, specific for the definitive chicken heart.

As far as the negative reactions obtained in the animals which had been sensitized with the heart tissues from 3 and 4 day embryos are concerned, we propose that they are explained not by the absence, but by the negligible amount of organospecific antigens present in the heart tissues at these stages of development. In connection with this, an experiment was conducted in which the guinea pigs were sensitized with heart tissues from embryos incubated 3 and 4 days in a dose 4 time as large as the sensitizing dose used in the preceding series of experiments (64 mg instead of 16 mg).

On the 21st day after the sensitizing injection, the animals were desensitized to species-specific antigens. All the animals reacted very strongly to the injection of serous proteins. Therefore, in order to preserved the animals, the desensitization was done in two doses (2 intraperitoneal injections of 500 mg apiece, 6 hours apart).

The results of this experiment are shown in Table 2. The guinea pigs which had been sensitized with heart tissues from embryos incubated 3 days did not respond to the injection of the water-salt extract from the definitive chicken heart tissue by a positive anaphylactic reaction. However, a positive anaphylactic reaction

TABLE 1

Anaphylactic Reaction in Guinea Pigs Sensitized With Suspensions of Heart Tissue From Chicken Embryos in Response to Injection of Serum and Extract From Adult Chicken Heart

Sensitization. Antigen—suspension of heart tissues from embryos (16 mg)	Reacting injection. Antigen—chicken serum		Test for complete desensitization. Antigen chicken serum (300 mg)	Reacting injection. Antigen—extract from adult chicken heart tissues	
	Dose in mg	Reaction	Reaction	Dose in mg	Reaction
Incubated 3 days	250	-|-|-	—	1000	—
The same	250	-|-|-|-	—	1000	—
" "	250	-|-|-|-	—	1000	—
Incubated 4 days	250	-|-|-	—	600	—
The same	250	-|-|-	—	600	—
" "	250	-|-|-	—	600	—
" "	250	-|-|-|-	—	400	—
" "	250	-|-|-	—	400	—
" "	250	-|-|-|-	—	400	—
Incubated 6 days	250	-|-|-|-|-|-			
The same	500	-|-|-|-	—	400	-|-
" "	500	-|-|-	—	400	-|-
" "	500	-|-|-|-	—	400	-|-
Incubated 8 days	500	-|-|-	—	400	-|-|-
The same	500	-|-|-	—	400	-|-|-|-
" "	500	-|-|-	—	400	-|-|-
" "	250	-|-|-|-|-			
Incubated 18 days	500	-|-|-	—	400	-|-|-
The same	500	-|-|-	—	400	-|-|-
" "	500	-|-|-	—	400	-|-|-
Incubated 12 days	250	-|-|-|-|-			
The same	500	-|-|-	—	400	-|-|-
" "	500	-|-|-|-	—	400	-|-|-
	500	-|-|-	—	400	-|-|-
Incubated 16 days	500	-|-|-|-	—	400	-|-|-
The same	500	-|-|-	—	400	-|-|-
" "	500	-|-|-|-	—	400	-|-|-
Incubated 19 days	500	-|-|-	—	400	-|-|-
The same	500	-|-|-	—	400	-|-|-
" "	500	-|-|-|-	—	400	-|-|-|-
Suspension of adult chicken heart tissues	500	-|-|-	—	400	-|-|+
The same	500	-|-|-|+	—	400	-|-|-|-
" "	500	-|-|-|-	—	400	-|-|-|-
.	300	—	.	400	—
.	300	—	.	400	—
.	300	—	.	400	—

Note: + tremor, rubbing nose and ears, dishevelled fur, panting, slight temperature decline; ++ the same symptoms, but more pronounced, the guinea pig often sneezed; +++ the same symptoms, still more sharply pronounced; convulsive kicks, cough, lying on side, but animal lived; ++++ all the symptoms very acute, animal died; - no symptoms of anaphylactic shock; · no injection.

TABLE 2

Anaphylactic Reaction in Guinea Pigs Sensitized With Suspensions of Heart Tissue From Chicken Embryos in Response to Injection of Extract From Adult Chicken Heart Tissue

Sensitization. Antigen—suspension of heart tissues from embryos (64 mg)	Desensitization. Antigen—chicken serum (1,000 mg)	Test—completed desens. Atg. 300 mg ser.	Reacting inject. Atg. 600 mg extr. adult chicken heart tissues
	Reaction	Reaction	Reaction
3 day incubation	++	—	—
The same	+++	—	—
» »	++	—	—
4 day incubation	+++	—	+
The same	+++	—	+
» »	++	—	+

was observed in the animals which had been sensitized by heart tissue from 4 day embryos.

Therefore, the results of this experiment showed that the heart tissues of 4-day embryos contain a small quantity of organospecific antigens, but that there are evidently no organospecific antigens of the definitive heart contained in the tissues of the 3-day embryos. Therefore, one can assume that organospecific antigens appear in the heart tissues on or after the fourth day of incubation.

It was interesting to note that, as our histological studies showed, the morphological structure of the heart undergoes a series of essential changes in the embryos incubated 4-6 days, which confirms the observations of earlier authors (see Lillie, 1952). After the fourth day of incubation, the transformation process of the tubular heart into the four-chambered heart begins; the cardiac septa are formed (interventricular and interauricular), and the syncytial-cellular structure of the myocardium characteristic of the tubular heart changes into the syncytial-muscular structure. At this time, the laterally striated, definitive myofibrils begin to appear in the myocardium.

As Table 1 shows, the anaphylactic reaction was weakly expressed in the guinea pigs which had been sensitized by the suspension of heart tissues from embryos at the 6th day of development. A well-expressed anaphylactic reaction was observed in the guinea pigs which had been sensitized by the suspensions of heart tissues from embryos at later stages of development. The experiments showed that antigenic organospecificity increases greatly as the embryo develops.

Analogous data were obtained from the ring-precipitin reaction (Table 3). The tissues of the developing chicken heart possessed antigenic species-specificity at all the stages of embryogenesis studied. Organospecific antigens, characteristic of the definitive chicken's heart, were only found in the tissues of the developing heart after the 4th day of incubation, but then the antigenic organospecificity increased as the embryo developed.

Therefore, as a result of our studies, we have established that the heart tissues possess a well-expressed antigenic species specificity at all of the developmental stages studied. The organospecific antigens typical of the definitive chicken's heart evidently only appear after the fourth day of incubation. The appearance of the organospecific antigens precedes the fundamental morphological changes in the developing heart which occur after 4 days of incubation.

TABLE 3

Results of Ring-Precipitin Reaction Between Serums Against Chicken Blood Serum and Against Adult Chicken Heart Tissues and Antigens of Embryonic Organs at Various Stages of Development.

Serums	Antigen dilution	Antigens of embryo tissues												Antigens of adult chicken tissues		
		4-day incubation	8-day incubation				12-day incubation			16-day incubation					
		Heart	Heart	Liver	Heart	Liver	Heart	Liver	Spleen	Heart	Liver	Spleen	Heart	Liver	Spleen
Against chicken blood serum (No. 725)	1 : 50	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	1 : 100	++	++	++	++	++	+++	+++	+++	++	++	++	++	++	++
	1 : 200	+	++	+	++	++	++	++	++	++	++	+	+	++	+
	1 : 300	−	+	+	+	+	++	+	+	+	+	+	+	+	+
	1 : 500	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Against chicken heart tissues (No. 1059)	1 : 50	−	++	−	+++	−	+++	−	−	+++	−	−	++++	−	−
	1 : 100	−	+	−	++	−	++	−	−	+++	−	−	+++	−	−
	1 : 200	−	−	−	+	−	+	−	−	++	−	−	+++	−	−
	1 : 300	−	−	−	−	−	+	−	−	+	−	−	+++	−	−
	1 : 500	−	−	−	−		−	−	−	+	−	−	++	−	−
	1 : 1 000						−			−			+	−	−
	1 : 2 500												−		
Normal (rabbit No. 725 before immunization)	1 : 50	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1 : 100	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1 : 200	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Note: ++++ heavy ring, precipitate formation; +++ strongly expressed ring; ++ definite ring; + weakly expressed ring; - no ring.

SUMMARY

Antigenic properties of the developing chicken heart were followed up through different stages of embryogenesis, the methods of anaphylactic reaction, desensibilization and precipitation being used. At all stages of development, tissues of the developing heart manifested significant antigenic species-specificity. Organ-specific antigens peculiar to the definitive chicken's heart appear for the first time at the fourth day of incubation, and rise significantly with the development of the heart. Appearance of these antigens coincides with the beginning of morphological transformation of the tubular heart into the four-chambered one.

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ON THE INFLUENCE OF THE CENTRAL NERVOUS SYSTEM ON CELL DIVISION

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Recently, the role of the nervous system in the regulation of the regenerative processes and its influence on the proliferative processes and on the origin and growth of new formations has been the subject of many works. Since the processes of cell division are closely associated with these complex biological phenomena, one can suggest that nuclear and cell division depend on the condition and function of the nervous system.

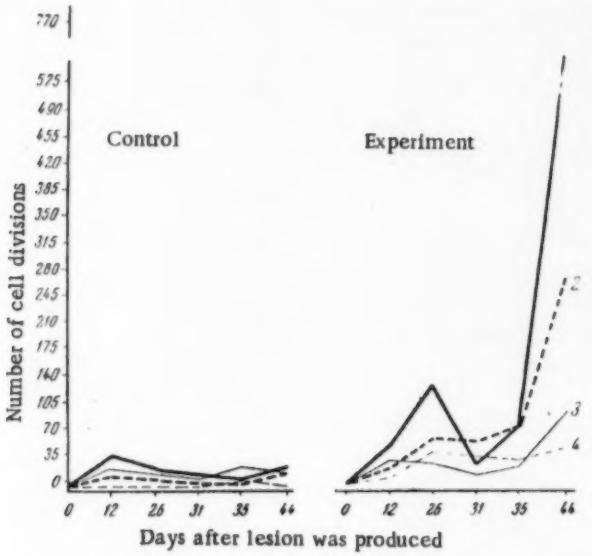

Figure 1. Intensity of cell divisions during healing of experimental lesions in dog No. 23. 1) number of mitoses in epidermis; 2) number of amitoses in epidermis; 3) number of mitoses in connective tissue; 4) number of amitoses in connective tissue.

As early as 1880, M. D. Lavdovsky, studying the morphological changes of salivary gland secretory cells caused by long stimulation of a tympanic string by induction current, showed that, in this case, the number of mitoses in the cells increased.

In recent years, works have appeared in which the authors made tests to approach the study of cell division

processes from new directions, taking into account the functional condition of the entire body and especially, the influence of the nervous system [1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13].

However, the way in which the nervous system influences cell division processes is still not clear. According to some authors [9], the number of mitoses decreases under conditions of denervation, while, according to others [7], denervation intensifies cell division.

Studying the effect of spinal cord transection on the healing processes of cutaneous lesions in dogs [13], one author points out the sharp change in cell division activity in connection with the broken nerve links. This observation was the basis for a more profound study of the central nervous system's influence on the processes of cell division.

The spinal cord in dogs was completely transected at the level of the V lumbar vertebra, thereby disturbing the connection of the hind legs with the central nervous system.

A lesion was experimentally induced on one of the denervated legs. A similar lesion was made on the front leg, which served as the control. Material for histological examination was resected at different intervals – from the 1st to the 79th day. The pieces were fixed in 10% neutral formalin and imbedded in celloidin. Hematoxylin combined with eosin, picro-indigo-carmine or picrofuchsin, iron hematoxylin or a combination stain of iron hematoxylin and Mallory's mixture were used as the stains. 36 dogs were operated upon and clinically observed. Material obtained from 25 animals was histologically processed. The cells in a dividing condition were counted in material obtained from 5 dogs. Beginning the 4th day after the lesion had been induced and ending the 44th day, the mitoses and amitoses were counted on 5 sections at each stage of the wound process. Both in the experiment and in the control, from 20 to 80 sections from each animal were examined individually, sections of the epidermis, its derivatives, and the adjoining connective tissue. The lack of any difference in the rate of cell divisions in the normal skin of the anterior and posterior extremities and in the tissues of the lesions in these extremities, not preliminarily denervated, was established by special observation.

Analysis of the data resulting from the cell division computations showed that the average ratio of the number of mitoses in the tissues of the control to the number of experimental lesions fluctuated from 1 : 2 to 1 : 18; the ratio of amitoses, from 1:10 to 1:98. In the connective tissue, this ratio fluctuated from 1:1.5 to 1:6 for mitoses and from 1:8 to 1:66 for amitoses (see Table 1).

Figure 1 shows the curves of cell division intensity characterizing the changes at the early stages of healing. In the control lesions, the number of dividing cells increased considerably until the 12th day, when they gradually decreased until the 31st day, i.e., until the lesion was completely epithelialized, to the normal physiological level. In the experimental lesion, mitotic and especially amitotic activity of the cells increased steadily according to the stage of the wound process, showing the active epithelial proliferation.

Figure 2, a shows the epidermal outgrowth, with 8 dividing cells visible in the field of vision. Mitotic forms were found in both the horny cells of the epidermis and in the marginal cells of the basal layer on top of the epithelial wedge (Fig. 2, b).

The data presented clearly show that the number of dividing cells in both the epidermis and the connective tissue was much greater in the lesion cut off from the central nervous system than in the control lesion, and that the greatest increase was in the number of amitoses.

In the experimental lesions, along with the usual amitotic forms found on the majority of sections, massive fragmentation was usually found, which led to the formation of multinuclear elements, having from 3 to 12 nuclei (Fig. 3).

It should be particularly noted that denervation causes more active cell division in the epidermis and its derivatives than in the connective tissues. Dividing cells appeared in the epidermal derivatives, although, normally, they occur there extremely rarely (for example, the glandular and myoepithelial cells of the sweat glands).

Along with the increased number of cell divisions in the tissues of the denervated lesion, signs of epidermal simplification and of a disturbance in the correlative relations of the epithelium and connective tissue appeared rather early. The epidermis lost its specific differentiation; its cells, especially in the horny layer, increased in size and stained less intensely, and their cytoplasm became cloudy. The intercellular spaces grew

TABLE 1

Change in Cell Division Intensity Under Conditions of Denervation

No. of animals	Epidermis						Connective tissue					
	Mitoses			Amitoses			Mitoses			Amitoses		
	Control	Experiment	Ratio	Control	Experiment	Ratio	Control	Experiment	Ratio	Control	Experiment	Ratio
3	132	835	1:6,3	21	456	1:21,7	63	203	1:3,2	8	61	1:7,6
6	213	408	1:1,9	15	213	1:14,2	49	191	1:3,8	2	16	1:8,0
23	59	1 041	1:17,6	6	588	1:98,0	32	196	1:6,1	2	132	1:66,0
24	109	589	1:5,4	23	607	1:26,4	34	62	1:1,8	3	23	1:7,6
30	146	747	1:5,1	8	76	1:9,5	56	76	1:1,3	2	19	1:9,5

Figure 2. Numerous mitoses in epidermal outgrowth of skin (a). Dog No. 23, skin of experimental leg 44 days after production of lesion. Objective 20, ocular 7x; b) mitosis in cell of basal layer on top of epithelial wedge. Dog No. 23, skin of experimental leg, 26 days. Objective imm. 90, ocular 10 x.

Figure 3. Nuclear fragmentation in horny layer of epidermis. a, b, c) in dog No. 23, experimental leg, 34th day. Objective imm. 90, ocular 15 x; d)- skin of experimental leg in dog No. 24, 29th day. Objective imm. 90, ocular 10 x.

much larger, and the epidermis became reticulated in places. One could often see huge nuclei, two or three times as large as ordinary nuclei, in the epithelial sheet. Active epithelial proliferation and disappearance of the basal membrane caused infiltrative growth of the epidermis, whose cells formed tongue-like forms which penetrated considerably deeply into the underlying connective tissue (Fig. 4). Some epithelial cells pulled away from the epithelial wedges and became much larger in size with a large nucleus and a weakly-staining, foamy cytoplasm.

As a working hypothesis, one may propose that,

when there are more mitoses than amitoses in denervated lesions, the epidermis grows infiltratively (a shaft of mitotically dividing eells perpendicular to the surface of the skin), which causes it to grow into the underlying connective tissue in tongue-like forms. When there are more amitoses than mitoses, the epidermis only thickens, and does not develop the typical infiltrative growth.

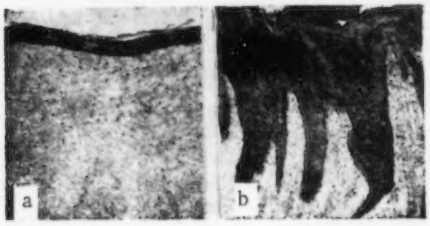

Figure 4. General view of vertical cross-section of lesion. Dog. No. 23, 26th day. Objective 8 x, ocular 7 x. a) control; b) experiment.

In any case, there is no doubt that sectioning the spinal cord causes a great increase in the number of cell divisions during the regeneration of cutaneous lesions.

The data presented allow the assumption that the central nervous system has a regulating effect on cell division, and that, when its impulses are excluded, the amitotic activity especially, as well as mitotic activity of the cellular elements are disinhibited.

That the number of dividing cells increased when the spinal cord of a dog was cut, as occurred in the experiments described, agrees with results obtained on axolotls by N. A. Ioff, who, although he did not study cell divisions directly, came to the same conclusion on the basis of observations on the healing of wounds.

SUMMARY

The effect of section of the spinal cord on cell division in the tissues of cutaneous lesions has been studied. Observations of 25 myelotomized dogs have shown, that in case of cutaneous lesions disruption of tissue innervation caused augmentation of cell division and intense infiltrative growth of the epidermis. These phenomena are connected with correlated disturbed relations of the epithelial and connective tissues.

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MORPHOLOGY AND PATHOMORPHOLOGY

CHANGES OCCURRING IN THE CARDIAC NERVOUS APPARATUS DUE TO THE ACTION OF ULTRA-HIGH-FREQUENCY FIELD

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Exactly how the changes caused by the action of an ultra-high-frequency (UHF) field on the body are effected is still not clear. Some researchers believe that the changes occurring in the organs are caused by the specific action of the UHF field itself, while others believe that they are caused by the diathermic effect of this factor. The role played by the nervous system in the pathological reactions which arise in the organs due to the action of the UHF field has not been explained. The available data from morphological studies do not well support the physiological facts known concerning this subject.

As an example, we cite the studies which have been made regarding the effect of UHF waves on the heart. From the works of N. V. Tyagina (1957), the action of these waves is known to cause serious changes in cardiac activity. Long exposure to a UHF field is attended by structural changes in the myocardium [10]. It is possible that these structural changes of the myocardium cause functional disturbances in the activity of the organ. With equal basis, one could propose that these disturbances are caused by changes in the nervous apparatus of the heart, which has great influence on the functional regulation of the organ. However, this important problem has not yet been resolved. In general, there are only a few works which study the morphological changes occurring in the nervous system under the influence of the UHF field, and nothing is known about the effect of the UHF field on the peripheral parts of the nervous system.

The above-mentioned was our reason for studying the condition of the cardiac nervous apparatus in animals subjected to the general action of a UHF field. We examined the nerve centers participating in innervation of the heart and the innervated substrate – the myocardium – as well as the intraorganic nervous apparatus.

From the works of E. K. Plechkova [12] and of A. Ya. Khaborova [13], it is known that the cardiac receptors consist of the endings of neurons whose bodies are located in the nodose ganglia of the vagus nerve and in the upper thoracic spinal ganglia (Th_1 - Th_5). Sympathetic innervation of the heart is realized through the nerve cells of the lateral nucleus of the intermediate zone of the eighth cervical and the first two thoracic segments of the spinal cord (central neurons) and by the cells of the sympathetic cervical ganglia (peripheral neurons). Parasympathetic innervation of the heart is represented by the cells of the dorsal nucleus of the vagus nerve in the medulla oblongata, branches of which cells terminate in pericellular apparatuses on the neurons of the cardiac ganglia.

EXPERIMENTAL METHODS

All these formations were examined. The medulla oblongata, the spinal cord, and the ganglia of the

spinal cord, the sympathetic trunks and the vagus nerves were stained by Nissl's method (alcohol fixation with celloidin imbedding). In order to examine the intraorganic nervous apparatus of the heart, pieces of the heart were fixed in 12% neutral formalin and then impregnated with silver by the Bielschowsky-Gross method.

The myocardial sections were stained with hematoxylin-eosin, for fat, and according to Heidenhain.

The first experiments were done on four male cats, which were subjected to the general action of a UHF field for 2 to 10 hours (for 2 hours a day).*. The body temperature was observed to rise in the two animals which had been exposed to the action of a UHF field with a current density of 0.03 w / cm^2 in power during the experiment; in two other cats, a less intense influence of the field (with a current density of 0.005-0.01 w / cm^2 in power) did not cause a hyperthermic condition. The animals were sacrificed 72 hours after the end of the experiment.

EXPERIMENTAL RESULTS

Examination of the preparations showed that some components of the intraorganic cardiac nervous apparatus had undergone serious changes. This was particularly true of the myelinated fibers and the sensory endings.

Although a certain number of unchanged myelinated fibers were found, a rather large number of changed ones were also observed. The most frequent manifestations of change were coarsening and increased impregnation of the fiber, bulges and neuroplasmic incrustations on it. Such fibers lost their usual structure. The axon, medullary sheath and neurilemma – the components of a normal fiber – became indistinguishable, and the entire fiber stained diffusely with silver to an intense black.

Such a picture is not specific for the action of a UHF field. Similar pictures were observed by B. I. Lavrentyev [8], D. N. Vyropaev [1], V. F. Lashkov [10] and V. V. Kupriyanov [5] on pathological material, found by B. A. Dolgo-Saburov [2] and V. V. Kupriyanov [4] in experiments with oxygen starvation and experimental uremia [6], and observed after the action of roentgen rays on the body [7].

In some fibers, intensely impregnated portions alternated with weakly impregnated portions. Such fibers had uneven, bulging contours, and very often those sections where the impregnation was poorly absorbed were the most swollen. This type of change is illustrated in Fig. 1. V. V. Kupriyanov mentions similar changes when describing the results of experiments with oxygen starvation.

The most profound changes appeared in the preterminal sections of the receptor fibers. As Fig. 1 shows, it was there that the most pronounced bulges and neuroplasmic incrustations were observed and that "myelin balls" appeared, evidently caused by the rupture of the neurilemma. The changes observed confirm the view of B. A. Dolgo-Saburov and V. V. Kupriyanov that the preterminal sections are the most "woundable" parts of the peripheral branch of an afferent neuron.

In contrast to the myelinated fibers, most of the nonmyelinated fibers retained their normal structure.

Profound changes were also observed in the sensory endings. As is known, there are two main types of cardiac receptors: receptors with constricted arborization, which are characterized by the concentration of the receptive substance, and diffuse receptors with wide-spread branches. According to the data of A. Ya. Khabarova, the first type is made up of the endings of vagus nerve sensory fibers, while the second belongs to the afferent neurons of the spinal ganglia.

Although a certain number of the sensory endings remained intact, many of the receptors examined were changed. Sharp changes in the preterminal sections, i.e., heightened argentophilia, and the breaking off of the reticula the "little rings" and the "little knobs" from the branches of the receptor fibers, pointed to the occurrence of irreversible degenerative processes in some of the cardiac sensory nerve endings (Fig. 2). Such changed endings were found in all layers of the organ's wall.

Both types of receptors were changed in all four of the experimental animals, so that it was impossible to say that one type of receptor was more "woundable" than the other; some sensory endings, however, changed in a different way from other sensory endings. In the receptors with the concentrated receptive substance, the

* The experiments were done on male cats in order to exclude the changes which B. A. Dolgo-Saburov [3] observed in the cardiac nervous apparatus of female cats during pregnancy.

preterminal sections stained an intense black with silver, became swollen and formed varicosities and neoplasmic incrustations. The receptor fiber terminals were also extremely argentophilic. Some of the receptor end structures kept their connections with the terminals, while others lost it (see Fig. 2).

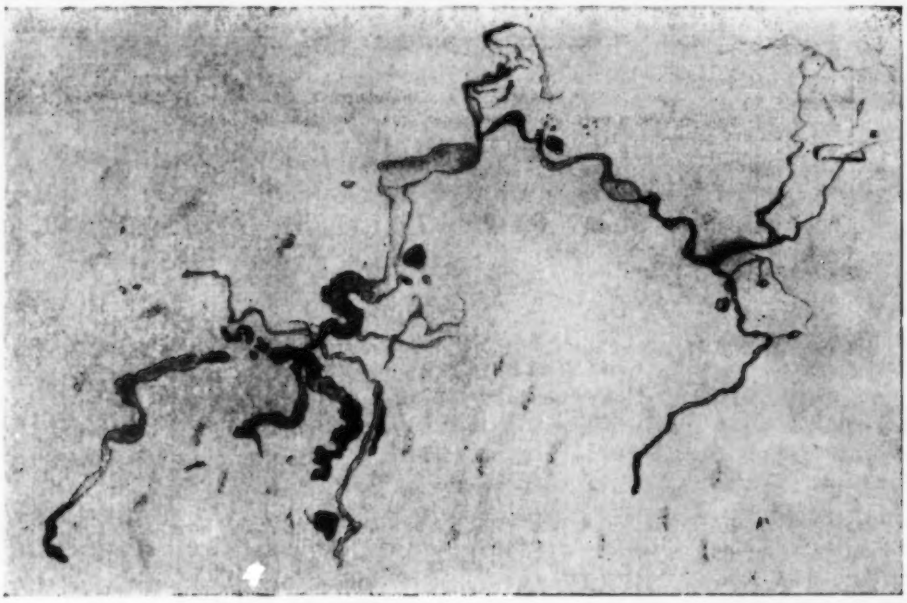

Figure 1. Changes in the preterminal sections of the receptor fibers in the wall of the left auricle of a cat. Intense action of the UHF field for 10 hours. Magnif. ocular 15x, objective 40 x. Impregnation by Bielschowsky-Gross method.

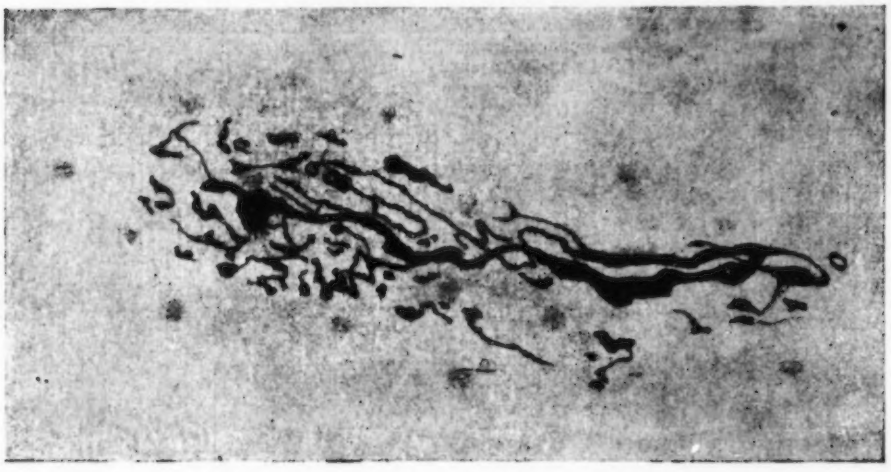

Figure 2.
Degeneration of a receptor with constricted type of branching in the wall of a cat's right auricle. Intense action of the UHF field for 2 hours. Magnif. ocular 15 x, objective 40 x. Impregnation by Bielschowsky-Gross method.

Figure 3.
Destruction of a Receptor with diffuse type of branching in the wall of a cat's right auricle. Intense action of the UHF field for 10 hours. Magnif. ocular 15 x, objective 40 x. Impregnation by Bielschowsky-Gross method.

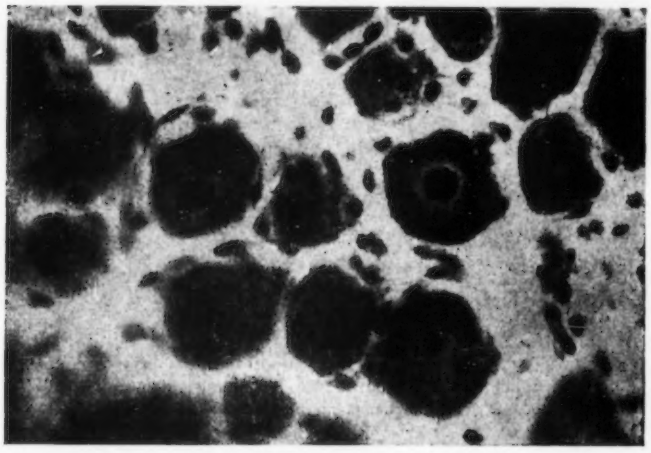

Figure 4.
Group of irreversibly changed nerve cells of spinal cord ganglia (Th_1) of a cat. Intense action of the UHF field for 10 hours. Photomicrograph. Nissl's stain. Magnif. ocular 15 x, objective 40 x.

Figure 3 shows a more or less typical view of a changed receptor with the diffuse type of branching. Heightened argentophilia was also observed here, although no neuroplasmic incrustations nor swelling of the receptor fiber or its terminals could be seen. The destructive processes are the most evident – the breaking off of the terminal structures and the disintegration of the whole length of the sensory ending. This heterogeneity in the change pictures existing in the different types of receptors is explained, we believe, by the difference in the amount of receptive substance contained in the sensory endings with the diffuse type and in those with the constricted type of arborization.

The next thing which interested us was the condition of the nerve cells in the ganglia of the heart and their pericellular apparatuses. A large majority of the nerve cells looked as usual, and only very rarely did one find single elements with signs of reactive changes. Slight changes were also observed in the pericellular apparatuses. The changes consisted in the heightened argentophilia and occasionally in the swelling of the pericellular apparatuses. These pictures seem identical with those which B. I. Lavrentyev [9] observed in a live frog's heart which had been subjected to the action of UHF waves.

No substanital pathological change was observed when the condition of the cardiac muscle was examined.* The muscular tissue was normal, and slight changes, which can frequently be found in practically healthy animals, were found on a small piece from only one animal.

Therefore, examination of the heart showed that individual elements of its nervous apparatus (myelinated fibers, receptors, pericellular structures) became changed, sometimes very greatly. These changes were evidently partly reversible and partly irreversible. However, the UHF field intensity which we used did not cause noticeable structural deviations from the normal in the substrate innervated by the nervous apparatus.

The next section of this work will examine the extraorganic nerve centers which participate in the innervation of the heart. After finding changes in the nervous apparatus of the organ, one would expect to find changes in the sensory cells of the spinal ganglia and of the vagus nerve ganglia. And, in actuality, among the cells of the spinal cord ganglia, we did find some which were changed. However, one must point out immediately that there were not many of these changed specimens; the majority of the cells had a normal nucleic and cytoplasmic structure.

The changes in the sensory cells of the spinal ganglia were for the most part reversible and were mainly concerned with the chromatophilic matter – redistribution of its granules and partial chromatolysis. More profound changes in the cell structure indicating the death of the neuron were less often found. This was observed in the two animals which had been subjected to the more intense action of the UHF field. Figure 4 shows a group of the cells. The nuclei of two cells are intensely stained; the chromatophilic matter has lost its usual granular structure, and chromatolysis has occurred in the region of the perikaryon. There was no doubt that the change in these neurons was irreversible.

A completely different picture was found upon examination of the sections from the nodose ganglia of the vagus nerve; the only reaction changes observed were slight structural displacements of the chromatophilic matter in individual cells. Nor were changes found in the sympathetic cervical ganglia or in the dorsal nucleus of the vagus nerve in the medulla oblongata. Only the cells of the spinal cord were further affected by the action of the UHF field. In one cat, a diffuse and thorough staining of the dendrites was observed in some cells, and the axon was beginning to stain thoroughly. In the posterior horns, separate cells stained intensely and had angular contours. The dense staining extended quite a distance along their branches. In another animal, an infiltration of the brain tissue by elements of the blood was observed in the intermediate zone. In these cats, single cells with signs of irreversible changes were also found in the intermediate zone of the spinal cord.

Therefore, the experiments conducted showed that the action of a UHF field of a definite intensity first causes changes in the afferent neurons. In the vegetative innervation centers of the heart and in its peripheral components, there is either only very slight change or none at all.

Besides the material described, sectional material from two dogs which had been subjected to the action of a UHF field in the experiments of N. V. Tyagin for many months (two hours daily with a current density of 0.005-0.01 w / cm^2 in power) was at our disposal.

There were no changes observed in the cardiac nervous apparatus of these animals. Although a large number of receptors were examined, no changed forms were found. Small varicosities and sometimes the

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* The myocardial preparations were kindly examined by D. S. Sarkisov, Doctor of Medical Sciences.

heterogeneous impregnation of axons could be observed. There was a remarkable development of the connective tissue in the ganglia. In addition, various cell elements in the spinal ganglia and in the nodose ganglia of the vagus nerves showed signs of death – residual nodes of Nageotte and single "empty" capsules, some filled with blood elements. The cells of the spinal cord were also changed. We observed changed cells most frequently in the lateral nucleus of the intermediate zone, in the Clarke-Stilling columns and in the posterior horn's own nucleus. Some of the cells in these nuclei had thoroughly stained interstices and perinuclear or marginal hyperchromatosis. The nerve cells retained their normal structure in the medulla oblongata and in the sympathetic cervical ganglia.

We should like to point out two aspects of the picture – the fact that a considerable number of cardiac receptors were intact and that residual nodes were present in the ganglia of the vagus nerves and in the spinal ganglia. These facts are in complete accord with the data presented above. Therefore, in the first place, the changes found in the receptors of the first four animals, i.e., the cats, did not lead to the degeneration of all the sensory endings. The majority of them were retained and became definitely adapted to chronic UHF field influence. In the second place, the degeneration of the receptors, or nerve cell endings, did not always imply the death of the nerve cell to which they were attached. New receptors were formed by regenerative processes occurring in the preserved portions of the neurons. Some of the neurons, however, did die, which was shown by the degeneration of the receptors after the 2-10 hour exposure, the irreversibly changed nerve cells in the ganglia of the spinal cord and of the vagus nerves in these same animals and by the fact that chronic exposure caused the appearance of residual nodes in the respective ganglia. All this shows that the afferent neurons are extremely sensitive to the action of high frequency waves.

It was interesting to compare the morphological data which we have presented with data from experimental clinical studies on the effect of the UHF field on cardiac activity. N. V. Tyagin observed bradycardia, changes in the S-T interval and a thickening of the descending slope of the R wave after intense exposure of the animals (dogs). With chronic intense action of a UHF field, the above electrocardiographic changes disappeared, and adaptation phenomena were observed. This data was obtained by N. V. Tyagin from experiments made on the two dogs already mentioned in this work, material from which we examined.

Involuntarily, an analogy arises with the results of the morphological studies. Structural changes of the cardiac nervous apparatus occurred in the animals which had been given a comparatively small dose of exposure (0.005-0.01 w / cm^2), but there were no definite indications of change in the nervous apparatus of those dogs in which adaptation to UHF field action was observed. The structural changes caused by the action of the UHF field may possibly bring about the functional shifts which N. V. Tyagin described in the operation of the heart.

Naturally, the question of why these changes occur arises – are they the result of general hyperthermia? Our data does not give a definite answer. However, one must remember that two out of the four cats were exposed to the UHF field action at a distance far enough from the emanation source to preclude the occurrence of a hyperthermic condition , and that changes nevertheless occurred in these animals, in both the cardiac receptors and separate cells of the spinal ganglia. One can therefore propose that the structural changes in the nerve tissue are caused by more than general hyperpyrexia of the body.

Analyzing the changes caused in the body by the action of a high frequency field, A. V. Lebedinsky [11] suggested that some of these changes could be explained by the stimulation of the afferent nerves.

The comparative lack of material makes any more definite conclusion impossible. One can, however, conclude that the changes occurring in the peripheral parts of the nervous system, and particularly in its afferent elements, must be taken into account when studying the effect of a UHF field on the body. It may be that some of the pathological reactions taking place in the body are not primary, and are not caused directly by UHF field action, but by the changes in the afferent links of many reflex arcs.

I. P. Pavlov's principle that dynamics adapt to structure is especially important when the organ or tissue is subject to change. In this case, basic data can only be obtained by simultaneous examination of structure and function. Further investigation of the effect of a UHF field on the body should therefore be done by physiologists and morphologists working in close conjunction. Such a study would give a balanced and three-dimensional idea of the processes which this factor causes in the body.

SUMMARY

The afferent neurons of the heart were the first to be affected in cats subjected to the action of ultra-high-frequency field from 2 to 10 hours. The receptor fibers of sensory endings, mainly the preterminal sections were most severely damaged. Altered nerve cells were seen in the spinal cord ganglia (Th_1 - Th_3) and vagal nerve ganglia. No alterations were noted in the vegetative centers of the heart.

The assumption of an intense sensitivity of the afferent neurons to the chronic effect of the ultra-high-frequency field has thus been confirmed.

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RESTORATION OF AN OBLITERATED COMMON BILE DUCT IN DOGS

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(Presented by Prof. N. N. Zhukov-Verezhnikov, Active Member of the USSR Acad. of Med. Sci.

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Bryuno [1] and Klodnitsky [2], studying bilification in dogs with an exposed common bile duct, observed cicatrization of the duct in the region of the papilla in a series of cases.

When we studied bilification in dogs with the common bile duct exposed by I. P. Pavlov's method [3], we also observed cicatrization of the bile duct in the region of the papilla in three dogs.

Bile secretion from the exposed duct was observed in the dogs a few days after the operation. In all three dogs, a different intervals after the operation (4, 8, 10 months), the bile duct became cicatrized in the papilla region and bile secretion from it ceased. At this time, the scleras were jaundiced, the dogs' feces were colorless, and the urine was dark brown and looked like bile.

After a few weeks, the jaundice disappeared from the scleras, the feces and the urine regained their normal colors.

Two of the dogs were sacrificed and the other was left for further examination.

When autopsies were made on the dogs, pressure on the gallbladder in the region of the cicatrices caused normally colored bile to pour out into the duodenum. The whole length of the bile duct was greatly thickened, as is always observed in dogs with an exposed common bile duct.

Histological examination showed this duct to be rather wide and lined with a tall, prismatic epithelium (Fig. 1, a, Fig. 2). The circular and longitudinal muscle layers were well expressed.

At the place where the duct had been brought to the body surface, it was obliterated in the region of its papilla. The individual layers of the duct were undifferentiated and replaced to a considerable extent by connective tissue. Near the former orifice, the bile duct lumen, still differentiated in places, was filled with an abundant infiltration of small cells. Sharply changed, degenerating bile duct glands could be seen in the connective tissue surrounding the lumen of the duct.

One must note that the functional importance of the formations called bile duct glands is still not sufficiently clear. Some consider them to be abortive bile ducts, others, bile reservoirs, and a group of authors declare that their function is secretory.

Benediktov [1] describes the bile duct glands of dogs as broad, weakly-branching tubes, with lumina directed towards the duodenum.

In the region of the cicatrices near the duodenum, the bile duct looked like a large sac, lined with a tall, cylindrical epithelium with a cuticular rim. The mucosa on the inside of the sac was extraordinarily rich in crypts.

There was a well-expressed muscular layer with numerous bile duct glands in the wall of the sac. The muscular layer disappeared at the place where the epithelium lining the inside of the duct contacted the

* Deceased.

mucosa of the duodenum (Fig. 3).

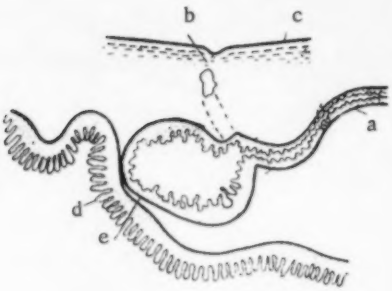

Figure 1. Schematic illustration of relationships in newly formed bile duct orifice. a) bile duct; b) bile duct brought up to skin surface; c) skin; d) mucosa of duodenum; e) newly formed part of bile duct.

Figure 2. Cross-section of a bile duct. Photomicrograph. Magnif. ocular 6×, objective 15 ×.

Histological examination permits the proposal that this is the place at which the bile enters the duodenum. We could not, however, find a clearly-defined bile duct opening. The bile duct glands also participated in the formation of the new bile duct near the duodenum, which was indicated by the presence of bile duct gland ducts directed towards the duodenum.

The possibility of the formation of a new pancreatic duct with a separate opening into the duodenum has often been mentioned in the literature. As I. P. Pavlov and G. A. Smirnov [4] have already described, this happens when the old duct is closed or ligated.

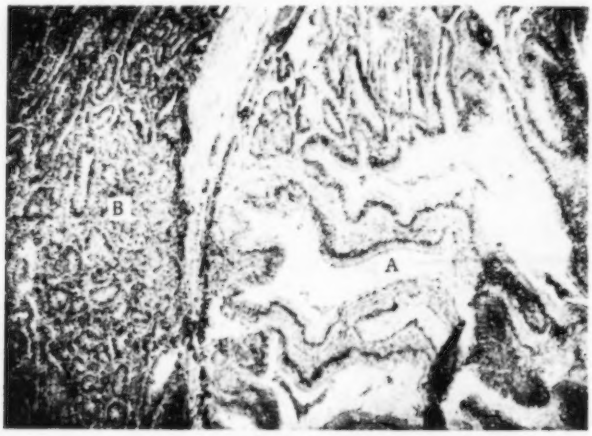

Figure 3. Newly-formed part of the bile duct. A) epithelium of the bile duct; B) mucosa of the duodenum. Photomicrograph. Magnif. ocular 6 ×, objective 15 ×.

We could not find any data on the formation of a new bile duct in the literature available to us.

The unique regenerative processes occurring in the body are indicated by these extremely interesting cases where new digestive gland ducts are formed.

SUMMARY

The construction of bile duct fistulae resulted occasionally in cicatrization of the duct in the papilla region. Autopsy of two dogs has demonstrated obliteration of the distal part of the duct and the formation of a new connection with the duodenum - restoration of the bile duct.

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PECULIARITIES OF HEMOPOIESIS IN THE SPLEEN AND LYMPHATIC GLANDS OF RABBITS

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When investigating the functions of hemopoietic organs, rabbits of various ages are used as experimental animals quite frequently.

The peculiarities at various ages that rabbits have in regard to the cellular components of the peripheral blood and their bone marrow have been detailed by many authors [1,3,5,9,10,11,12,13 and others]. Analogous data on the structure of lymphopoietic organs is available basically for adult animals [6,14,15] but this does not permit a complete analysis of blood formation at various ages in the life cycle of the rabbit. A more thorough investigation of the lymphocyte-forming organs is needed in the rabbit, moreover, as the peripheral blood of its young has a markedly lymphoidal character [5, 14].

EXPERIMENTAL METHODS

In the present study there was studied the cellular composition of the spleen and the lymphatic nodes of rabbits aged 1-2 $^1/_2$ months, the chinchilla breed being used. It is at this age that the rabbits usually cease nursing, change their teeth and acquire a complete furry coat [8]. Altogether 20 rabbits, weighing 400 to 900 grams and being maintained on a balanced ration, were examined. The animals were sacrificed by the air embolism method.

The spleen and lymph nodes were fixed in Zenker-formol and imbedded in paraffin or celloidin-paraffin. The sections were cut 4-5 μ thick, stained with hematoxylin – eosin, Romanovsky – Giemsa and by the Mallory method. Smears were fixed with methyl alcohol and stained with azure II – eosin. All conclusions were made after comparing the cytology of the smears with the character of the structure seen in the section.

EXPERIMENTAL RESULTS

The lymphopoietic tissues of the spleen in rabbits aged 1-2 $^1/_2$ months do not have germinal centers which are seen in the adult animals (Fig. 1). However, the general mass of lymphoidal elements and the relatively high cellular content of mitotic figures is testimony of the intense lymphopoiesis proceeding in this organ.

The lymphatic nodes in various regions of the body are characterized by unequal stages of differentiation. Thus, the mesenteric glands, as a rule, possess germinal centers within the follicles and multicellular marrow groups; while the knee, groin and axillary glands may actually exceed the mesenteric in size but are not as developed structurally. In them the lymphatic tissue forms small subcapsular structures bereft of centers of multiplication. Within the loops of the syncytium of the marrow portion a few lymphoidal elements are seen.

This absence of germinal centers within the splenic follicles and the lymphatic nodes of so many regions indicates the specific nature of the developing hemopoiesis. It is known that in man the splenic germinal centers are fully formed by age of two years [4], while the lymphatic nodes reach their full development by

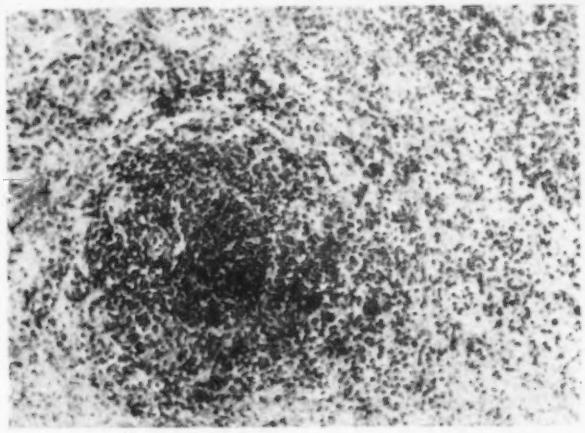

Figure 1. Malpighian body in 1 $^{1}/_{2}$ month old rabbit. Around the eccentric nutrient artery are concentrated mature, intensely staining lymphoidal elements which are surrounded by more juvenile forms. Between the zones of small and large cells is a narrow band of connective tissue. Stain: azure II – eosin. Magnification: ocular 7x, objective 20 x.

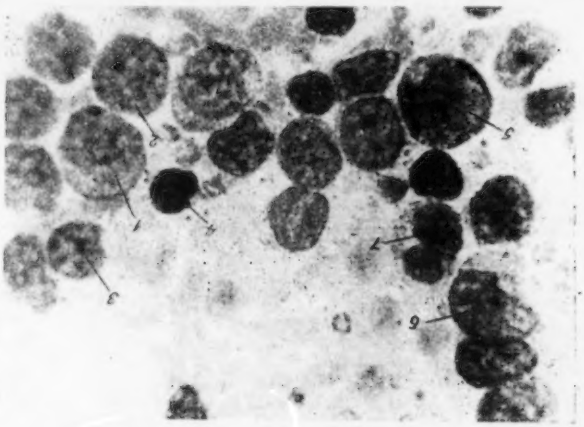

Figure 2. Elements of a splenic smear in a 2 month old rabbit. 1) lymphoblast, 2) prolymphocyte, 3) average lymphocyte, 4) small lymphocyte, 5) proerythroblast, 6) monoblast, 7) monocyte. Stain azure II – eosin. Magnification 7x, objective 90x.

ages 8 -12 years [2]. The most intense absolute and relative lymphopoiesis in the peripheral blood occurs in early childhood up to ages 2-4 years.

The follicular structure described above, void of germinal centers, demonstrates that rabbits at ages 1-2 $^{1}/_{2}$ months preserve some features of lymphopoieisis characteristic of animals in the embryonic stage and the earliest postnatal period. Further evidence of this type of hemopoiesis in the spleen is the low grade intensity of the erythro- and granulopoieisis (Table 1).

The dominant elements in spleen smears and lymphatic glands are cells of the lymphoidal series (Table 1, Figs. 2 and 3). In the spleen they are 91.99% while in the mesenteric glands – 94.5%.

The cells of the erythrocytic and granulocytic series in all stages of differentiation within the spleen are only 4.05 %. In the lymphatic glands only mature pseudoeosinophiles and eosinophiles are present and their total number corresponds (0.35%) to the level of these elements in the peripheral blood [5].

The other cells of the splenic smear (3.91%) and lymphatic gland (5.4%) are reticular elements, hemohistioblasts, hemocytoblasts, macrophages and cloudy cells.

Judging by the juvenile and mature forms of the monocytes, in the spleen and lymph glands of rabbits aged 1-2 $^{1}/_{2}$ months monocytopoiesis goes on (Fig. 2).

In the lymphatic glands are seen all transitional phases from the plasmablast to plasmatic cells (Fig. 3).

The ratio between mature and juvenile forms of the lymphoidal series is different in young and adult rabbits. Thus, in rabbits of 2 $^{1}/_{2}$ years, the undifferentiated elements compose 0.7% of the lymphoidal series of the spleen, and the mature compose 65% [14]; in rabbits 1-2 $^{1}/_{2}$ months old. These figures are correspondingly 2.22% and 89.77%. This is confirmed by the data furnished by the partial lymphogram and by maturation indexes. The maturation index of lymphoidal cells of rabbits 1-2 $^{1}/_{2}$ months old is 0.027 (Table 2), but in adult rabbits it is 0.011 [14].

The somewhat larger number of juvenile elements in the lymph glands as compared with the spleen may be partially explained by the great amount of blood in the spleen, the blood containing more mature lymphocytes.

The number per 1000 of cells undergoing mitosis in the spleen smears from the rabbits being studied averaged 4 with the fluctuations being between 2 and 8 while in the lymphatic glands – 6 with fluctuation range being 0 to 10. As a rule, they were juvenile forms of the lymphoidal series – lymphoblasts and prolymphocytes. It seems quite possible that many of the mitotic figures were hemocytoblasts, this possibility having being raised by N. D. Strazhenko and D. N. Yanovsky [7].

In the cells of the lymphatic series in young rabbits, amitoses are exceedingly rare (1 amitosis per 1000 cells in the smear with fluctuation between 0 and 2). In the spleen, normoblasts are seen at times dividing amitotically, while in the lymph glands the same can be said of plasmatic cells.

TABLE 1

General Cytograms of the Spleen and Mesenteric Lymph Glands in Rabbits Aged 1-2 1/2 Months

Elements	Spleen		Lymphatic nodes	
	Arithmetical mean	limits of fluctuation	Arithmetical mean	Limits of fluctuations
Large lymphoidal reticular cells	1.03	0.2—1.6	1.4	0.8—2.0
Small lymphoidal reticular cells	0.46	0.2—0.6	0.4	0.2—1.0
Hemohistioblasts	0.14	0—0.4	0.25	0.2—0.4
Hemocytoblasts	0.31	0.2—0.6	0.4	0.2—0.6
Lymphoblasts	0.91	0.4—1.2	1.35	1.0—1.6
Prolymphocytes	1.31	0.4—2.4	1.95	1.4—2.4
Lymphocytes				
Broad protoplasm	5.06	1.2—8.2	2,95	2.0—4.0
Average	51.83 } 89.8	49.6—55.2	47,7 } 90,9	42.8—54.2
Small	32.77	27.6—37.8	40,3	29.2—47.4
Plasmablasts	—	—	0.2	0—0.4
Plasmacytes	—	—	0.95	0.4—1.4
Plasmatic cells	0.54	0—0.8	1.3	0.6—2 2
Monocytes	0.74	0.4—1.2	0,3	0—0.6
Macrophages	0.23	0—0.8	0.1	0—0.3
Promyelocytes, variously granulated granulocytes	0.11	0—0.2	—	—
Pseudoeosinophiles	3.31	1.8—4.6	0.25	0—0.4
Eosinophiles	0.23	0—0.6	0.1	0—0.2
Basophiles	0.14	0—0.6	—	—
Proerythroblasts	0.06	0—0.2	—	—
Basophilic erythroblasts	0.2	0—0.6	—	—
Polychromatophilic erythroblasts, Normoblsts	0.4	0.2—0.8	—	—
Cloudy cells	0.2	0—0.8	0.1	0—0.2

TABLE 2

Partial Lymphocytograms of the spleen and Mesenteric Lymph Glands in Rabbits Aged 1-2 1/2 Months

Elements	Spleen		Lymph glands	
	Arithmetical mean	Limits of fluctuation	Arithmetical mean	Limits of fluctuation
Lymphoblasts	1.0	0.8—1.6	1.6	1.4—1.8
Prolymphocytes	1.5	0.6—2.2	2.2	1.4—2.4
Broad protoplasm lymphocytes	5.8	1.4—9.4	3.35	2.2—4.0
Average lymphocytes	55.4	51.8—59.2	50.4	45.4—51.8
Small lymphocytes	36.3	30.8—42.6	42.45	41.2—48.2
Maturation index	0.027		0.039	

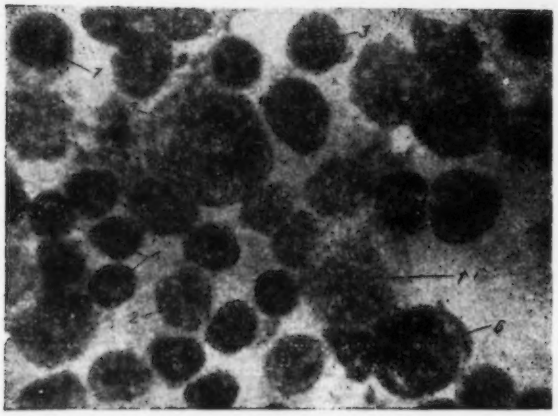

Figure 3. Elements of a mesenteric gland smear from 2 month old rabbit. 1) lymphoblast, 2) broad protoplasm lymphocyte, 3) average lymphocyte, 4) small lymphocyte, 5) plasmablast, 6) plasmacyte, 7) plasmatic cell. Stain azure II – eosin. Magnification ocular 7x, obj. 90x.

SUMMARY

The structure and cellular composition of the spleen and lymph glands of young rabbits were studied. The juvenile features of hemapoiesis in animals of ages 1-2 1/2 months were noted and the differences between the various lymph glands were stressed. As compared with the adult, many more immature cells were seen.

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FUNCTIONAL AND MORPHOLOGICAL CHANGES PRODUCED BY PROFOUND GENERAL REFRIGERATION IN THE CENTRAL NERVOUS SYSTEM AFTER THE HEART HAS BEEN EXCLUDED FROM THE GENERAL CIRCULATION

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For successful intracardiac surgery it is essential to develop safe methods permitting exclusion of the heart from the circulation so that its interior can be operated on under direct vision in a dry field. However, when this is done, blood no longer reaches the tissues and, unless special precautions are undertaken, within 5-7 minutes irreversible changes occur in the central nervous system.

An enormous aid in maintaining tissue stability during oxygen starvation is a general cooling of the body below temperatures of 28-30°. This procedure has been employed lately during intracardiac operations both experimentally and in the clinic [2,3].

However, the method of general refrigeration and exclusion of the heart has not been well-developed in the clinic and is by no means a safe procedure. The pathological and physiological changes which occur in the nervous system (which is most sensitive to anoxic interference) have not as yet been investigated adequately. There is as yet no absolute answer as to how long the heart may be excluded from the circulation while the patient is in the refrigerated state. The aim of the present study, undertaken jointly by a pathological physiologist, surgeon and neuropathologist, was to outline the precise procedures of generalized refrigeration to be used in a surgical clinic.

EXPERIMENTAL METHODS

All experiments were done on dogs. The animals were always cooled while narcotized (morphine, penthotal, ether and curare– like drugs– diplatsin, paramion*). The best results were obtained under ether anesthesia with oxygen combined with morphine and curare-like drugs. To cool the animals either cloth-covered bags filled with ice or ice baths (water temp. 2-4°) were used. Cooling was stopped when the body temperatures fell to 28-26°. During surgery, the temperature was usually lowered another 2-3°. Cooling was maintained from 40 minutes to 2 hours. The temperature was depressed most rapidly when the animal was immersed in water. To exclude the heart from the circulation the superior and inferior vena cava were clamped off, the unpaired vein being ligated.

EXPERIMENTAL RESULTS

6 control experiments were done with cardiac exclusion at normal temperatures. In 3 animals where the heart was excluded for 4 $^1/_2$ to 6 $^1/_2$ minutes, on the day following surgery, when the effect of the anesthesia

* Russian trade name.

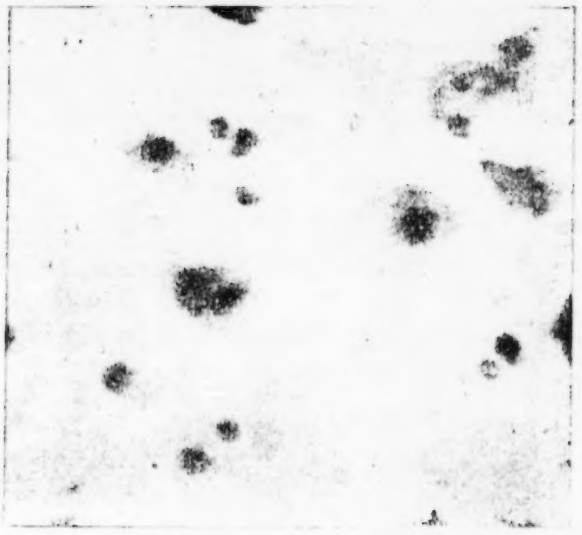

Figure 1. Severe alterations in cortical cells of dog cerebral hemisphere when animal had been subjected (without refrigeration) to the heart being excluded from the circulation for 7 $^{1}/_{2}$ minutes. Thionin stain (after Nissl). Ocular 12, obj. 40.

had worn off, the first severe changes in the nervous system could be seen to proceed, the behavior of the animals could not be distinguished from the normal. When the heart had been excluded from the circulation for from 7 to 7 minutes 45 seconds (3 experiments) 2 dogs perished; one – from cardiac failure during the circulatory exclusion period, the second – 5 hours after the exclusion, the animal never emerging from a deep comatose state. The surviving dog for 2 days was fearful, aggressive, permitted no approach and bit the individual feeding it. By the 3d day these disturbances subsided and on the 4th day it was sacrificed for histologic studies. The data obtained on cardiac exclusion is comparable with that obtained by I. P. Petrov [1].

The results of the refrigeration experiments are shown in the table.

Experimental Results When the Heart is Excluded from the Circulation During General Cooling.

Length of time of cardiac exclusion from the circulation in minutes	Survived			Perished				Total
	Observed 7 days to 6 months	Sacrificed within first 7 days in good condition	Total	During cardiac exclusion	Within 1st day after surgery	Later	Total	
To 10	1	—	1	2	1	—	3	4
10—14	2	—	2	3	3	—	6	8
15—19 $^{1}/_{4}$	4	8	12	2	7	2	11	23
21—26	—	—	—	—	3	—	3	3
Total	7	8	15	7	14	2	23	38

The high mortality was due to various complications, principally disturbances of the cardiovascular system. During the course of our experiments, we discovered effective prophylactic and therapeutic measures which markedly reduced the mortality. Out of the first 15 dogs, 12 perished, while in the last 7, in spite of

prolonged cardiac exclusion, only 2 animals died.

Up to the moment of cardiac exclusion the refrigerated animals were, as a rule, in a state of inhibition whose depth was about equal to the usual narcotized level.

During cardiac exclusion the severe hemodynamic alterations were most conspicuous. The arterial pressure dropped precipitately but not to zero, in most experiments being 10-20 mm mercury, this being apparently associated with preservation of some arterial tone. The venous pressure rose soon reaching the arterial level.

In the central nervous system there could be observed an increasing inhibitory state. The vessel tone diminished and the arterial pressure fell, the pupil in most instances dilated, respiration in many instances ceased although sometimes they assumed an agonal character which would subside when the clamps on the caval arteries were released. If, before clamping, corneal, pupillary and spinal reflexes were present, within the first few minutes they would disappear.

When vessel tone was preserved, removal of the vein clamps would quickly restore the arterial blood pressure. However, more often special measures were required to restore vessel tone and cardiac activity.

It must be remarked that in some experiments, on the background of restored reflex activity, the blood pressure remained at 40-50 mm mercury pressure, the medication for restoring tone gave only a transient effect and the animal would perish 5-8 hours after the operation. At autopsy, there would be usually found a large hemorrhagic pleural exudate or a secondary pneumothorax. It may be assumed that in some instances there must have occurred a special type of shock stage produced by the profound inhibition of the central nervous system after the cardiac exclusion, as well as the influence of the cooling, besides, possibly, ether overdosage as its blood solubility increases with decreasing temperature.

As the nervous system would resume functioning after the operation, first of all it was vessel tone that was restored, then spontaneous respiration, pupillary reaction to light and corneal reflexes, increased tone of the muscles of the extremities, reappearance of the tendon reflexes, sometimes manifestations of plastic tonus, shivering of the extremities, then the animal would raise its head, response to sound, light and pain, would become manifest and, finally, the functions of standing and walking would return. The day following surgery the animals would behave normally.

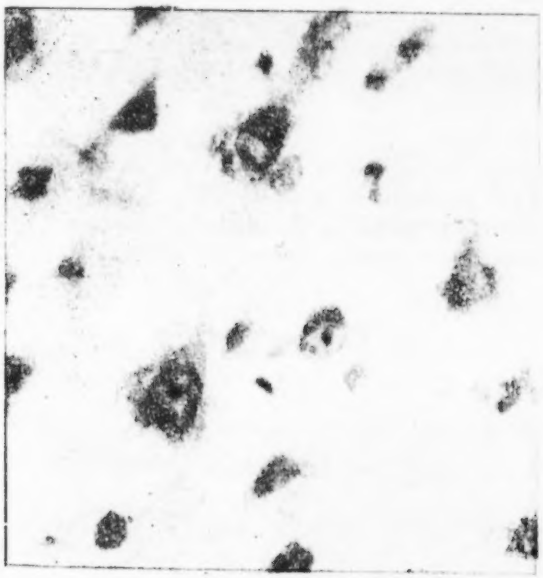

Figure 2. Reversible changes in nerve cells (honeycombed structures in the protoplasm) of the cerebral cortex observed after the heart had been excluded from the circulation for 19 minutes after the animal had been refrigerated. Thionin stain (after Nissl). ocular 12, obj. 40.

Thus restoration, as a rule, would occur within the first day and would be complete.

In a whole series of instances some functions would not return by following the "ladder" switching in procedure of including the various divisions of the nervous system according to their phylogenetic age. Thus, usually the first reflex to be restored was the pupillary reaction to light (midbrain) and only later the corneal reflexes (medulla oblongata), spinal reflexes, etc..

The morphology of the nervous system was examined in 20 animals. In the control animals, whose heart had been excluded from the circulation for 6 $^{1}/_{2}$ - 7 minutes while the body was at normal temperature, there were marked circulatory disturbances (leukostasis, erythrostasis in the vessels, fragmentation of the vessel walls), brain edema, hemorrhages, nerve cell alterations characteristic of severe disease (Fig. 1) up to transformation into "ghost-cells", ischemic cells, hydropic nerve cells and disappearance of entire groups of nerve cells. There could be observed swelling and wrinkling with chromatolysis and hyperchromatism of the chromatophilic substance and appearance of honeycombed and basophilic structures within the bodies of the nerve cells.

The refrigerated dogs, even when subjected to much longer cardiac exclusion (up to 19 minutes), showed many fewer alterations. Basically, the changes seemed to be of a reversible type: scattering of the chromatophilic substance, its redistribution within the bodies of the nerve cells, honeycombing of the protoplasmic structure (Fig. 2), manifestations of a moderate hyperchromatosis, moderate edema of the nerve cell bodies and their outgrowths and some circulatory upsets. There were also irreversible changes: severely altered nerve cells, "ghost cells", ischemic nerve cells and nerve cells which had disappeared.

In all instances studied the severest alterations were seen in the cerebral cortex of both hemispheres, less in the hippocampus, cerebellum, and still less in the subcortical ganglia, the lower olives, nuclei of the cranial nerves and spinal cord. The nerve fibers and their endings were altered less than the cell bodies. In the early stages, the neurologist could see only some degenerative changes in the astrocytes. In the later stages (several days and later) there could be observed an increase in the glia with an overgrowth of its protoplasm.

The neurological data and the pathological-morphological investigations show that, after cardiac exclusion, the functional alterations in many instances were less than the morphologic changes. This might be due to the great plasticity of the nervous system, this allowing functional restoration even after such severe damage that some nerve cells actually become destroyed. Restoration is also accomplished by the reversibility of many of the changes.

Our results show that general body refrigeration combined with narcosis increases the stability of the nervous system when subjected to anoxia and thus prevents severe irreversible functional disturbances even when the heart is excluded from the general circulation for as long as 15-19 minutes.

SUMMARY

At normal body temperatures, exclusion of the heart from the circulation for as long as 7 minutes is apt to lead to irreversible changes and death (two out of three). When the body temperature is lowered to 24-28°, even 19 minutes of heart exclusion from the circulation produces mostly only reversible central nervous system alterations. Usually, by the second postoperative day, no functional changes of the nervous system could be observed.

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METHODS

AN EXPERIMENTAL METHOD OF STUDYING THE KOROTKOV SOUND PHENOMENON

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In spite of numerous studies the precise mechanism giving rise to sound effects when an artery is compressed has not been fully clarified.

To aid in doing this we have developed a method which shows the dependence of the sound effects upon the degree of artery compression and the manner in which the blood passes by the narrowed portion of the vessel.

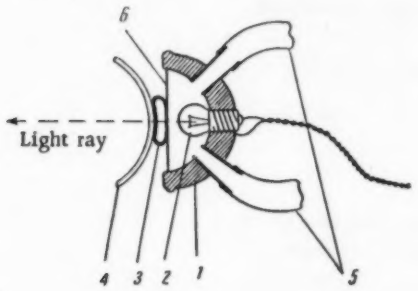

Figure 1. Diagram of the method employed for the simultaneous observation of sound effects and the manner of blood passage through the compressed portion of the artery (in experiments with local compression of a prepared artery). 1) body of bell stethoscope; 2) light; 3) artery; 4) watch crystal; 5) tubes of stethescope; 6) membrane of stethoscope.

EXPERIMENTAL METHODS

The experiments were performed on dogs under evipan narcosis. The apparatus is shown schematically in Fig. 1.

In an aperture made within the head of an ordinary stethoscope an ordinary lamp from a pocket flashlight was placed. This stethoscope was placed under the artery which had been previously dissected free of surrounding connective tissues. The artery would be compressed by the convex surface of the watch crystal against the celluloid membrane of the stethoscope. When the lamp was lighted the pulsating blood waves were readily seen while simultaneously there could be heard the sounds caused by the arterial compression.

When the artery was tightly compressed so that the systolic pressure and the elastic resistance of the arterial wall were exceeded, the lumen of the artery became completely occluded and it looked in the transmitted light like a transparent bright rose ribbon. The blood waves struck against the compressed portion of the artery but no sounds could be detected.

As the compression was slowly relaxed, occasional spurts of blood would pass during systole through the compressed artery to its distal portion, this being accompanied by the appearance of arterial sounds.

As the external pressure was diminished, larger and larger amounts of blood would be seen passing during systole through the compressed area. The sound tones became more intense and other sounds began to appear so that it became difficult to differentiate the initial tones in the general sound complex. However, it could

be seen that until such time as the diastolic pressure was being exceeded the arterial lumen remained compressed during diastole so that blood passed the compressed portion in spurts and that the sounds heard in the stethescope coincided with them.

When the external compression became less than the diastolic pressure, the lumen of the artery no longer remained totally compressed during diastole; the artery lost its resemblance to a transparent ribbon and stayed filled with blood during diastole. Blood would then reach the distal portion of the artery not in spurts but as a continuous stream, its size and speed being magnified with each systole.

In the stethoscope could be heard only sounds arising from the acceleration of the blood stream passing through the narrowed portion of the artery.

The relationships just noted can be clearly illustrated by simultaneous objective recording of the sound effects(with aid of microphone) and of the passage of the blood through the compressed portion of the artery (with aid of photoelements).

The method of this objective registration is shown schematically in Fig. 2.

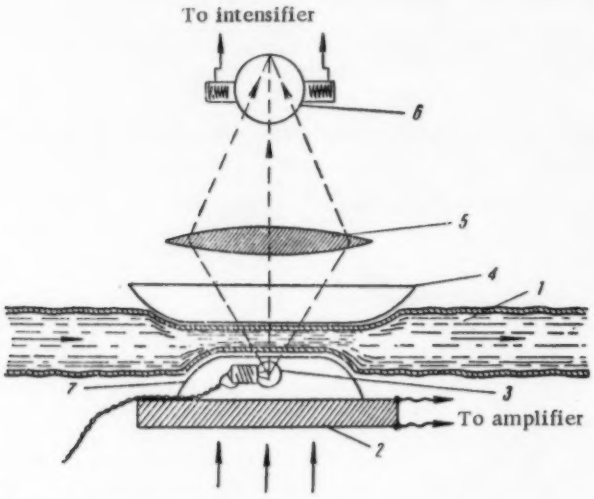

Figure 2. Diagram for objective recording of arterial sounds and the passage of blood through its compressed portion. 1) artery (arrows show direction of blood flow); 2) crystal piezomicrophone with watch glass (7) glued to it; 3) lamp for illuminating the arterial lumen (3 volts); 4) plexiglass arterial compressor; 5) objective for focusing light on the sensitive layer of the photoelement; 6) antimony cesium photoelement. The three arrows underneath indicate direction of the pressure upon the arterial lumen compressing it.

The dissected artery was placed on top the watch glass glued to the crystal piezomicrophone which served to record sounds. Under the watch glass was placed the light from thestethoscope remaining connected to its battery. The artery was pressed against the watch glass by a piece of transparent plexiglass beyond which was placed a vacuum antimony – cesium photoelement impenetrable to light, in a shielded case with an objective and diaphragm. The light from the lamp passes through the artery and diaphragm and is focused by the lens on the sensitive layer of the photoelement. Passage of some blood during systole through compressed portion of the artery altered the intensity of the light beam thus leading to fluctuations of the current passing through the photoelement, these becoming intensified and being recorded on the oscillograph. Simultaneously, the other needle recorded the sound effects within the artery which were taken up by the piezocrystal and intensified by the 3 – cascade amplifier. Altering the arterial compression enabled us to register clearly the sound effects

within the artery and the passage through its compressed portion of the systolic blood portions during the different stages of decompression, as can be seen on the curves (Fig. 3).

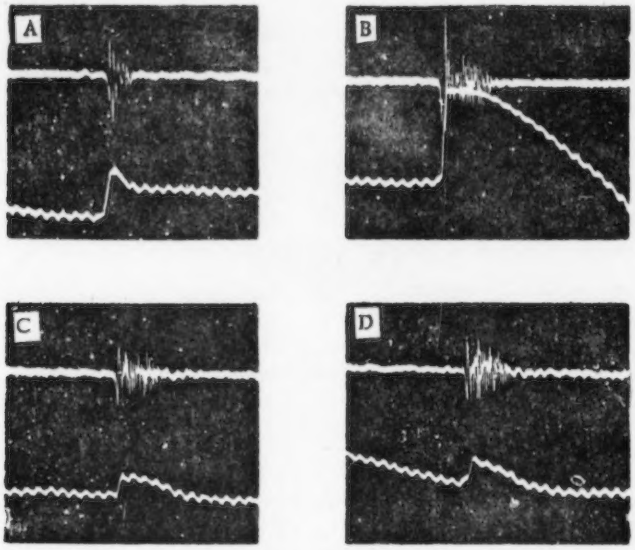

Figure 3. Registration of sound effects and the passage of portions of blood through the compressed portion of the artery when a local area is squeezed during a short experiment on a dog. Upper record – arterial sounds recorded by piezomicrophone. Lower tracing – blood passage through the compressed artery as recorded by the photoelement. Frequency of photoelement recording – 50 periods per second. Rapidity of drum movement – 107 mm/sec. Magnification two times. A) During external pressure just in excess of systolic. During systole a little blood penetrates by the compressed area. In the artery some tone arises. B) during external compression approaching the diastolic. During systole much blood passes by the compressed area. At beginning of stroke against arterial lumen wall a sound is created. During subsequent passage of blood by the compressed area noises arise. C – D) during external pressure below diastolic. Arterial lumen during diastole remains patent. During systole it dilates somewhat. Blood flow by the compressed area is uninterrupted. During systole there is only some acceleration of blood streaming by the compressed area this being accompanied by appearance of noises within the artery.

EXPERIMENTAL RESULTS

The procedures described enabled us to establish that tones and noises arise in the compressed artery for different reasons. The tone arises not, as supposed by many investigators, at moment the pulse wave strikes the obstruction but after a portion of the blood has passed by the obstruction and impinged on the blood mass (and arterial wall) below the compressed area. The tone represents short harmonic (periodic) oscillations of relatively high amplitude.

The noise arising in the mass of blood accelerated by the systolic stroke and hurrying it by the compressed portion of the artery is a series of aperiodic oscillations of different frequencies and amplitude, lasting much longer than the tone. Such oscillations are typical for whirling (turbulent) fluid movements caused by acceleration of the stream velocity.

This objective method has aided us in clarifying somewhat disputed points relating to source of sounds within the artery when its lumen is compressed, the blood pressure being investigated by the Korotkov method.

SUMMARY

A simple method for studying the Korotkov sound phenomena is described and diagramed. The sounds and blood flow can be placed in precise relation to each other. This enables one to demonstrate the precise relation of the passage of the blood stream to the origin of the sounds.

ARTIFICIAL BREEDING AND RAISING OF TADPOLES UNDER LABORATORY CONDITIONS*

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Amphibian tadpoles for a long time have been favorite objects of experimentation in various biological fields. Quite often there arises the need for having laboratory animals the year around and tadpoles of specific ages up to the stage of metamorphosis may be required to permit the work to go on.

There has been much work done describing evocation of artificial ovulation in frogs during the winter-time [2,5,6,8 and others], the effect of light has been investigated [3,4], temperature [1,3,9], rations on growth [3,8,10,12] and the development of the tadpoles of different species.

We have not recounted all the studies touching on the problem but nowhere have we seen a unified description of how to procure and breed frog tadpoles.

For the last two and one-half years, because we were studying some peculiarities of the ontogeny of Rana temporaria, we had to work with the tadpoles of this species during the fall winter and spring seasons. In the present communication we have combined our observations on 18 series of tadpoles bred in this laboratory.

EXPERIMENTAL METHODS

Oyulation was produced artificially by the method of Ya. M. Kabak [5]. In the October – January period the female received two injections (with an intervening day between them) into the subcutaneous lymph sac of a suspension taken from two frog hypophyses and then, 48 hours after the 2nd injection, artificial insemination was performed. In February one injection was sufficient while in March – one injection from a suspension taken from one hypophysis. In April and especially by May the female was ready to shed eggs naturally so that there was no longer any need for preliminary hypophyseal injections.

At the end of the period of natural egg laying, during June-September period, ovulation could no longer be induced. Living tadpoles can then be obtained no earlier than the middle of September.

When describing larvae, their age from the moment of fertilization may be used as a guide but with tadpoles differences are seen in succeeding series between tadpoles of the same age. As a consequence of this, it is more convenient to classify them by the various growth stages based on the identification of various internal and external structures. From the many classifications available [3,7,10 and others] the most practical is the one proposed by P. V. Terentyev [7]. This author describes a total of 33 stages from the unfertilized egg to the adult creature: further it is his grouping that we will use.

The fertilized roe, taken from one frog, was placed in two glass pans 5 cm deep with a diameter of 20-23 cm. The roe was covered with tap water which had stood for a day in the room so that temperature was

* Although this subject is well covered in previous publications, the editorial board has considered this work of sufficient practical merit to warrant publication.

14-16°C. From then on the pans were cleaned and the water changed daily. Until the 25-26th stage was reached (formation of gill slit and appearance of rear limb rudiments) the water level was maintained at 2 cm level, after which the level was decreased to 1.5 cm. From then on, less and less water was added and the pans were slightly inclined so that during metamorphosis the young frogs could crawl out "on dry land". During this period the pans were carefully covered with gauze cloth as otherwise the little frogs could readily get out and scatter.

For the first 2-3 days after fertilization the roe was left at room temperatures but then the pans were placed in a plywood enclosure forming a chamber with a door and one glass wall. For lighting and heating a 96 watt electric globe was kept within this chamber, this being switched off at night thus creating a night and day fluctuation both in light and temperature. The temperature of the water in the pans usually was about 15.5-17.5°C.

On the 7-10th day after fertilization (20th stage) the larvae having a general body length of 8.6-11.2 mm were redistributed into different pans, 20-30 tadpoles in each.

The tadpoles began to be fed at the 22-23rdstage (opening of mouth, appearance of gill fold and teeth). For the first 4-6 days they received only vegetation food: scrapings from aquarium walls, chips of vegetation but later ground meat was added to the food. During metamorphosis (29-30th stage) the tadpoles do not eat but at its end the young frogs can consume only small, live, moving food. During the fall– winter season it is difficult to obtain small insects and such so that many frogs are lost at this stage of development.

It should be underscored that for the first 30-35 days after fertilization very few of our tadpoles die. Also, in the still later stages our losses are minimal.

If the conditions enumerated above are watched (light, temperature, feeding, water level) it is quite feasible to have during the fall – winter season fully grown frogs at the end of 60 days, although, as a rule, the time needed is longer – 87 to 140 days.

Under natural conditions, metamorphosis in Rana temporaria begins between 50 and 180 days after fertilization, the Leningrad region being about 150 days [7]. So it can be seen that our results are comparable with the natural. The size of our tadpoles and young frogs also are same as seen naturally: our largest tadpoles – 40 mm, frogs – 9.6 to 14.5 mm; natural frogs measure 46 and 11 to 15 mm [8].

Our method requires a minimum of effort and has been proven on an adequate series to give living tadpoles.

SUMMARY

A simple method for artificial breeding of frogs, Rana temporaria, is described. The water must be kept at 15.5-17.5°C; illumination maintained for 8 hours daily; water depth in the pans be maintained at precise levels and vegetable and animal feeding be guaranteed as required.

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LEON ABGAROVICH ORBELI

ON HIS 75TH BIRTHDAY

Among the prominent physiologists of our country, the name of the Academician Leon Abgarovich Orbeli, Hero of Socialist Work, is one of the foremost.

A catholic scholar, a brilliant experimental worker, the founder of a large physiological school, L. A. Orbeli made a large contribution to the physiology of his native land and of the world through his own work and the work of his students.

The extensive and many-sided studies of L. A. Orbeli in the field of the physiology of the central and peripheral nervous system, sense organs, digestion, and urination made possible the establishment of new laws regarding the activity of these systems, suggested important theoretical principles which pioneered in physiology and were the basis for the solution of a number of practical problems.

Particularly noteworthy are the merits of Leon Abgarovich in the elucidation of the autonomous nervous system, and also in the creation of an evolutionary trend in physiology which permits the consideration of complicated functions of animals and man in the light of the theory of development.

L. A. Orbeli was born on July 7, 1882, in Erevan. After completing the Tbilis Gymnasium in 1899 he entered the Army Medical Academy. As a student, L. A. Orbeli joined the scientific profession. Under the direct guidance of I. P. Pavlov he carried out his first scientific work "The Comparison of the Work of Pepsin Glands Before and After the Cutting of the Vagus Nerves" for which he was awarded a Gold Medal by the Army Medical Academy.

After completion of the Academy, L. A. Orbeli worked in the laboratory of I. P. Pavlov and became one of his assistants in the elaboration of the new studies concerned with higher nervous activity.

In 1908 L. A. Orbeli defended his doctoral dissertation on "Conditioned Reflexes of the Eye of a Dog" and in 1909 he received, on the recommendation of I. P. Pavlov, a two-year appointment abroad. Working in the laboratories of prominent Western European scientists (Langley, Barcroft, Garten, Hering, and others) L. A. Orbeli became familiar with the works and methods of these scientists and carried out a number of experiments in various fields of physiology.

After his return from abroad, L. A. Orbeli again worked with I. P. Pavlov in the Institute of Experimental Medicine and in the Army Medical Academy. He assisted I. P. Pavlov in the direction of many collaborators who were developing the theory of conditioned reflexes.

Having become the director of the Physiological Laboratory of the P. L. Lesgaft Scientific Institute in 1913 and having become a professor of physiology I LMI in 1920, L. A. Orbeli began his independent work as a teacher of science. In this period he studied, with a number of collaborators, the problems of digestive glands, cerebrospinal coordination, the autonomic nervous system and others.

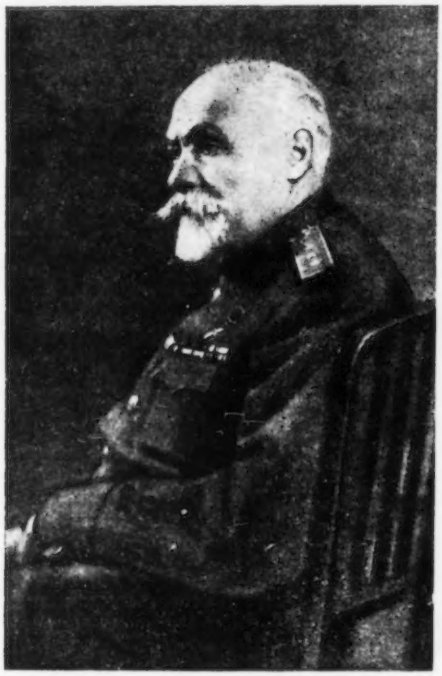
The scientific activity of Leon Abgarovich became particularly broad and fruitful in the various fields of physiology from the moment he became the director of a large group of collaborators in the Physiological Institute of the USSR Academy of Science and the Biological Station (later Institute of Evolutionary Physiology and Pathology of Higher Nerve Activity) in Koltusha.

In a short essay it is difficult to throw light upon the many-sided scientific activities of Leon Abgarovich. We shall, therefore, note only the principal landmarks of his creative path.

The first works of L. A. Orbeli in the laboratory of I. P. Pavlov were devoted to the study of conditioned reflexes from the visual analysor and the localization of the conditioned reflexes in the central nervous activity. He established the important fact that dogs do not differentiate colors and that conditioned reflexes are determined in dogs by changes in the light intensity; in his experiments involving removal of the upper half of the cerebral lobes in dogs new evidence was obtained that conditioned reflexes are a function of the cerebral cortex.

The work of L. A. Orbeli in the Pavlov laboratories (up to 1920) in the time of the burgeoning of the study of conditioned reflexes and his direct participation in the direction of collaborators in these laboratories enabled L. A. Orbeli to become one of the prominent workers in the field of higher nervous activity and to guide the elaboration of this study by a large number of collaborators. The results of the work on higher nervous activity and his own personal views in this field are presented in two monogtaphs "Lectures Regarding the Problems of Higher Nervous Activity" (1954) and "Problems of Higher Nervous Activity" (1949).

For many years Orbeli studied the problems of the physiology of digestion. Well known are his studies of the physiology of the pancreas (together with K. M. Bykov), of bile as the stimulant of the secretion of intestinal juices, of the properties of the enzymes of intestinal juices in man, of the physiological role of organic acids, and others.



From 1921 L. A. Orbeli began the elaboration of the problems of the physiology of the sympathetic nervous system. As a result of a large number of investigations he formulated a totally new concept about the basic functional principle of the influence of the sympathetic innervation on organs and tissues. Investigating at first the influence of sympathetic nervous fibers on skeletal muscles, and later on receptors and the central nervous system and various organs, L. A. Orbeli established the adaptational-trophic role of the sympathetic nervous system in the integration of all tissues and organs. The discovery of the influence of the sympathetic nervous system on the somatic formations of the organism, which was the source of the wide reputation of L. A. Orbeli, required a revision of the whole physiology of animal functions from this point of view. The establishment of the adaptational role of the sympathetic innervation was not only a contribution to the development of experimental and theoretical physiology but also had great meaning for medicine. The works of L. A. Orbeli and his collaborators formed an experimental basis for numerous empirical observations on the influence of the

sympathetic nervous system on trophic processes in the organism.

Of great theoretical value were the works of L. A. Orbeli concerning the study of the mechanism of cerebrospinal coordination. Proceeding from the conditioned reflex teaching of I. P. Pavlov, according to which it is possible to trace the rise of new reflex actions in a very short time, L. A. Orbeli advanced the view that the study of conditioned reflexes is the key to the elucidation of the development of reflex actions in general. In the early stages of the formation of conditioned reflexes, the cortex has a diffuse activity which is later replaced by the exact distribution of the centers of stimulation and checking in the cortex. In the opinion of L. A. Orbeli even the phylogenetically old sections of the central nervous activity (the stem region, the spinal cord) had to possess diffuse reactions from which, in the process of evolution, coordinated motor actions were developed. This was confirmed by the work of Kunstman and Orbeli which showed that by means of deafferentation of one extremity of the dog it is possible to expose the characteristics of diffusability of lower sections of the central nervous system. In general this diffusibility is not observed because the system of afferent fibers hinders the excessive spreading of stimulation in the spinal cord. Thus gradual analysis brought L. A. Orbeli to the conclusion that the highly developed central nervous system has remnants of old functional relations and that not only were in the process of evolution old functional relations definitively destroyed but that they were also suppressed by new formations of the central nervous system.

L. A. Orbeli's studies of the cerebellum have an important place in the work about the physiology of the central nervous system. Experimental work in this direction made it possible to see the cerebellum as the higher adaptational-tropic center and an aid to the large hemispheres of the brain. It was possible to demonstrate that the adaptational-trophic influence of the cerebellum extends to the digestive organs, the cardiovascular system, muscular functions, metabolism in tissues, etc. These influences are carried out by means of nerves as well as hormones.

Noteworthy are the studies of the effects of nociceptive irritations which were carried out in the laboratories of L. A. Orbeli.

The solution of this problem — in reality the problem of pain — has great theoretical and practical meaning. In this work the somatic syndrome brought about by a painful irritation was studied. In particular, the participation of the adrenals and the hypophysis in the painful reaction was established, the meaning of axon-reflector connections between the sections which perceive pain and internal organs, and the central nervous system was clarified. The results of this work are of exceptional interest to clinical medicine, especially in the treatment of nervous and internal diseases.

Important are also L. A. Orbeli's studies of the physiology of urination. Having worked out the methodology of the separate removal of the openings of the ureter in dogs, L. A. Orbeli, together with his collaborators, analyzed the nervous and hormonal mechanism of the activity of the kidneys and made important contributions to the theory of Keshin-Reberg.

The large body of factual material obtained in various fields of physiology was valuable to L. A. Orbeli in his plan to apply the ontogenetic principle to the study of physiological functions. This was distinctly shown even in his basic general work "Lectures on the Physiology of the Nervous System" (1935).

The data concerned with ontogenetic physiology accumulated in the preceding years gave L. A. Orbeli the opporunity to concentrate his attention in recent time on the elaboration of the problems of the evolution of functions.

In the newly-created I. M. Sechenov Institute of Evolutionary Physiology of the USSR Academy of Sciences, directed by L. A. Orbeli, work towards the solution of two fundamental problems is broadly conceived and carried out: 1) the study of the patterns and mechanisms of development of function in separate organs and organ systems and of the activities of the whole organism in the ontogenetic process, their complications, refinements, and modifications as a result of the influence of various external factors, and 2) the elaboration of general laws of functional ontogeny, which lead to the rise of complicated adaptation phenomena. According to L. A. Orbeli, " the study of the ontogenesis of function should not only elucidate the history of the perfection of physiological mechanisms which are capable of coping with the complications of the interactions of the organism with the environment and which provide an increase in the level of functional organization of animals in the ontogenetic series," but permit one also, "to broaden the scientific basis of medicine and to promote the working out of practical measures directed toward the heightening of the stability of the human organism in the new working

conditions created by contemporary technology."

During his whole creative life L. A. Orbeli knew how to combine a large amount of experimental scientific work with a large organizational, pedagogical and general activity. He directs large institutes and departments, fulfills the duties of Director of the Army Medical Academy, works in the presidium of the USSR Academy of Sciences, edits a number of biological journals, organizes meetings and conferences, etc. At present L. A. Orbeli is the president of the Central Soviet of the All-Union Society of Physiologists, Biochemists and Pharmacologists.

L. A. Orbeli has a significant part in the training of scientific personnel. His excellent abilities as a pedagogue and leader, the ability to place scientific personnel correctly, to take into account the personal inclinations of each of his students and to direct him properly has always attracted a large number of young scientific collaborators to his laboratory.

Among his numerous students a large number became prominent scientists, known for their work throughout our country.

His great erudition, his great ability to work, and his accessibility, ready willingness to help a scientific worker or a physician in the solution of some problem, his constant desire to share his knowledge and experience with others, gave L. A. Orbeli a well-deserved reputation in the scientific and medical world.

For distinguished service to the Soviet fatherland and to science the Presidium of the Supreme Soviet of the USSR has three times awarded Leon Abgarovich the Order of Lenin and other orders and medals. He was awarded the highest honorary title—a Hero of Socialist Work.

On the day of his 75th birthday L. A. Orbeli, leading a large scientific group, is full of creative projects for the solution of new problems in the field of physiology. As usual, his inexhaustible striving toils constantly for the good of his fatherland and of Soviet science. We wish Leon Abgarovich good health and great success on this noble path.

Professor A. A. Volochov

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE IN ENGLISH TRANSLATION

June, 1957

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RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Ekspti. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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